Semi-Automated Direct Colorimetric Measurement of Creatine Kinase Isoenzyme MB Activity after Extraction from Serum by Use of a CK-MB-Specific Monoclonal Antibody

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This semi-automated colorimetric assay for the MB isoenzyme of creatine kinase (EC 2.7.3.2) is based on a monoclonal antibody ("Conan-MB") specific for this isoenzyme and is a modification of a previously published method (Vaidya et al., Clin Chem 1986;32:657–63). A 0.64-cm bead coated with 2 to 3 μg of antibody is incubated with 100 μL of serum and 10 μL of 0.2 mol/L β-mercaptoethanol for 1 h at room temperature, to extract CK-MB. The beads are washed with de-ionized water and incubated with CK substrate for 45 min at 37 °C. A solution containing trans-1,2-diaminocyclohexane-N,N',N''-N'''-tetraacetic acid, p-iodonitrotetrazolium violet, and diaphragm is added and the resulting colored product is measured at 492 nm. The standard curve is linear to 200 U of CK-MB per liter, and analytical recovery is 97–113%. Total assay CV for low (9.7 U/L) and high (50.7 U/L) quality-control materials was 14.1% (n = 1878) and 11.6% (n = 1842), respectively. CK-MB activity correlated well (r = 0.978, n = 226) with CK-MB measured by a two-site mass immunoassay, and 99.4% of samples with CK-MB ≥12 U/L (n = 347) were verified by electrophoresis on agarose.

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The importance of measuring creatine kinase-MB (CK-MB, CK2, variant of ATP:creatine N-phosphotransferase, EC 2.7.3.2) as a diagnostic aid in identification of myocardial infarction has been well established.4 CK-MB is routinely measured in clinical chemistry laboratories worldwide by many methods, which vary in specificity, sensitivity, and precision (1–7). We recently published a method for the direct assay of CK-MB activity in serum based on the use of a unique monoclonal antibody ("Conan-MB") immobilized on latex particles 0.8 μm in diameter (8). The CK-MB was immunoeextracted from diluted serum samples, the latex beads were washed and separated by centrifugation, and then the catalytic activity of the bound CK-MB was measured. Although this assay was highly specific, manipulation of the latex particles was tedious, and precision was less than optimal.

We describe here our modifications of the assay that have made it suitable for routine laboratory use. The improvements include use of 0.64-cm (diameter) beads as solid support for the immobilization of antibody, use of undiluted serum, termination of the enzyme catalytic reaction by the addition of CDTA to chelate Mg2+, and the development of a

4 Nonstandard abbreviations: CDTA, trans-1,2-diaminocyclohexane-N,N',N''-N'''-tetraacetic acid; HISP, heat-inactivated serum pool; Bio-Tris, 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)-1,3-propanediol; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid; INT, p-iodonitrotetrazolium violet; TBS, Tris-buffered saline.

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colored product to increase the signal/noise ratio. The entire assay was semiautomated on commercially available equipment. We have monitored the performance of the assay in routine clinical use and compared results with those by a CK-MB mass assay and by electrophoresis.

**Materials and Methods**

Coating Beads with CK-MB-Specific Antibody

Production, characterization, and purification of a monoclonal antibody specific for CK-MB (Conan-MB) have been described previously (9).

The polystyrene beads (0.64-cm in diameter, with specular finish) were from Precision Plastic Ball Co., Chicago, IL 60641. We coated them with Conan-MB by passive adsorption. Ten thousand beads were washed five times with distilled water and once with 0.1 mol/L sodium phosphate, pH 6.0 (coating buffer). The beads were then covered with 1200 mL of a 42–50 mg/L solution of Conan-MB in coating buffer and incubated for 24–40 h at 4 °C, with occasional stirring. We removed the coating solution from the beads and assayed it for remaining protein by the method of Bradford (9), using the Bio-Rad Protein Assay Kit (Bio-Rad Labs., Richmond, CA 94804). To calculate the density of the antibody coated onto the beads, we multiplied the percent of protein bound to the beads by the original amount of protein and divided by the number of beads. Uncoated sites on the beads were blocked by incubation for 4 h at room temperature (or overnight at 4 °C) with Tris-buffered saline (TBS; 50 mmol of Tris, pH 7.2, and 150 mmol of NaCl per liter) containing 10 g of bovine serum albumin per liter. The beads were then washed five times with TBS and stored at 4 °C in TBS containing 10 g of bovine albumin and 0.2 g of sodium azide per liter.

Samples, Standards, and Quality-Control Material

Serum samples submitted to the Barnes Hospital or Jewish Hospital chemistry laboratories for analysis of CK-MB were stored at 4 °C and assayed within 24 h. β-Mercaptoethanol (final concentration, 15 mmol/L) was added to serum at Barnes Hospital immediately after separation from cells. Standards were prepared by adding purified CK-MB (10) to a serum pool that had been inactivated by heating at 56 °C for 45 min (heat-inactivated serum pool, HISP) and supplemented with 10 mmol of β-mercaptoethanol per liter. The value for the highest standard (S4) was assigned as 109 U/L after repeated assays for total CK based on a modified procedure of Rosalki (11) at 37 °C in a Flexigem™ centrifugal analyzer and with the manufacturer's reagent (Electro-Nucleonics Inc., Fairfield, NJ 07006). Threefold serial dilutions of S4 in HISP produced standards with CK-MB activities ranging from 4 to 109 U/L. Standards were divided into 0.5-mL aliquots and stored at −70 °C. Quality-control materials were prepared similarly to give CK-MB values of approximately 10 U/L and 50 U/L and stored in 1-mL aliquots.

Reagents

Creatine kinase reagent. The CK reagent used in the routine assay was prepared weekly by the chemistry laboratory of Jewish Hospital and stored at 4 °C. The final concentrations of components in the reagent were, per liter: 170 mmol of Bis-Tris, pH 6.8; 2.2 mmol of ADP; 10 mmol of magnesium acetate; 20 mmol of D-glucose; 2.9 mmol of NAD⁺; 4.8 mmol of AMP; 2.5 mmol of EGTA; 33 mmol of creatine phosphate; 2500 U of hexokinase (EC 2.7.1.1) from yeast; 1650 U of glucose-6-phosphate dehydrogenase (EC 1.1.1.49) from Leuconostoc mesenteroides.

Note that there is no sulfhydryl activator in this reagent. Enzymes, ADP, NAD⁺, and creatine phosphate were purchased from U.S. Biochemical Corp., Cleveland, OH 44122. Bis-Tris, AMP, and EGTA were from Sigma Chemical Co., St. Louis, MO 63178. Magnesium acetate and D-glucose were from Fisher Scientific, St. Louis, MO 63178.

Color/stop solution. This solution consists of 8 mmol of CDTA and 250 μmol of INT per liter of Tris buffer (160 mmol/L, pH 7.5). Stored at 4 °C and protected from light, the solution is stable for six months. Lyophilized preparations of "diaphorase" (EC 1.8.1.4) from Clostridium kluyveri were reconstituted in 50 mmol/L Tris, pH 7.5, to give an activity concentration of 2.5 × 10⁸ U/L and stored in aliquots at −20 °C. These were stable for at least one month. Diaphorase reagent is diluted 100-fold with CDTA/INT solution just before use. CDTA, INT, Tris, and diaphorase were obtained from Sigma Chemical Co.

Direct Colorimetric Assay of CK-MB

The routine assay as performed by the chemistry laboratory at Barnes Hospital is as follows:

1. Dispense 10 μL of 0.2 mol/L β-mercaptoethanol into wells of EIA Reaction Trays (Abbott Laboratories, Diagnostics Division, Irving, TX 75015).
2. Dispense 100-μL serum samples, standards, or quality-control materials, in duplicate.
3. Add one Conan-MB-coated bead to each well, and incubate for 1 h at room temperature on an American Rotator V platform shaker (American Dade, Miami, FL 33126) set at 150 rpm.
4. Wash the beads with 10 mL of de-ionized water, using a PROQUANTM™ Bead Washer and Reagent Dispenser (Abbott Laboratories).
5. Transfer the beads to EIA Assay Tube Racks (Abbott Laboratories) and add 250 μL of CK reagent to each tube via PROQUANTM.
6. Transfer the tubes to a 37 °C water bath and incubate for 45 min.
7. Add 800 μL of color/stop solution.
8. Mix, let the color develop for 5 min, then measure the absorbance at 492 nm with a Quantum™ II Dual Wavelength Analyzer (Abbott Laboratories).
9. Plot A₄₉₂ nm vs U/L value for each CK-MB standard.
10. Determine U/L values of quality-control materials and samples by comparison with linear regression analysis of the plotted standard values.

The assay is performed similarly at Jewish Hospital except that the Pentawash® II (Abbott Laboratories) is used to wash the beads and the Vector Photometer (Behring Diagnostics, La Jolla, CA 92037) is used to measure A₄₉₂ nm.

Alternative CK-MB Assays

We assayed 639 consecutive serum samples from Barnes Hospital by both the direct colorimetric assay as described above and by Enzygnost™ CK-MB assay (Behring Diagnostics), a two-site immunoenzymometric assay for measuring the mass of CK-MB; we followed the manufacturer’s procedure.

Over a six-month period, 347 serum samples with CK-MB values ≥12 U/L by the direct colorimetric assay were also assayed by an agarose electrophoresis method in which the activity of CK isoenzymes is measured semiquantitatively.
Potential interference from high concentrations of other CK isoenzymes was assessed by assaying HISP supplemented with CK-MB (26 U/L) with or without addition of CK-MM (15) or CK-BB (Calbiochem Behring Diagnostics, San Diego, CA 92112) at values ranging from 0 to 92 500 U/L.

Results
Optimization of Assay

We evaluated the utility of polyamide, polystyrene, and hydrazide beads, using the following techniques: passive adsorption before or after mild acid hydrolysis; covalent linkage via glutaraldehyde or carbodiimide bridges; activation by nitration followed by reduction and diazotization (16); linkage to commercially available hydrazide beads (Fierce Chemical Co., Rockford, IL 61103) directly according to the manufacturer's protocol or via protein A or goat antimouse-Fc antibody bridges. We assessed these different preparations by two criteria: (a) The mass of antibody adsorbed or bound to the beads was monitored by adding 125I-labeled ConA-MB to coating solutions and measuring the radioactivity associated with the beads after washing. (b) The amount of CK-MB extracted from solution by immobilized ConA-MB was determined by measuring both the amount of CK-MB enzymatic activity associated with the beads and the amount of activity remaining in solution. Monitored for 0–4 h, the extraction of CK-MB from solution was maximal after 2 h with all bead preparations, although the absolute amount of CK-MB extracted varied (Table 1). The amount of CK-MB activity extracted and that left in solution were reciprocal (data not shown), indicating that all CK-MB activity was accounted for.

The technique of passive adsorption to polystyrene beads produced the best extraction results (Table 1). Although under one condition we were able to covalently link 40 μg of ConA-MB onto a single bead, the amount and time course of CK-MB extraction with this preparation were not better than that attained with polystyrene beads with 2.5 μg of passively adsorbed antibody per bead. In fact, in most cases,

![Graph A](image1.png)

![Graph B](image2.png)

**Fig. 1.** Optimization of coating density of ConA-MB onto 0.64-cm (diameter) polystyrene beads for use in the direct assay of CK-MB. A: Beads were coated overnight at 4 °C in 0.1 mol/L sodium phosphate, pH 6.0, containing 2 to 128 μg of ConA-MB antibody per liter. B: Enzyme activity (A405 μm) bound by increasing amounts of immobilized ConA-MB after 1-h extraction at room temperature from HISP or added CK-MB (82 U/L) as measured after incubation of washed beads with commercial ConA reagent for 30 min at 37 °C.

### Table 1. Comparison of Different Coating Techniques for Immobilization of ConA-MB onto 0.64-cm Beads for Use in Direct Assay of CK-MB

<table>
<thead>
<tr>
<th>Coating technique</th>
<th>µg adsorbed</th>
<th>µg bound</th>
<th>Direct assay performance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Polystyrene beads</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adsorption</td>
<td>5</td>
<td>2.5</td>
<td>100.0</td>
</tr>
<tr>
<td>Poly-Phe-Lys + glutaraldehyde</td>
<td>80</td>
<td>7.0</td>
<td>104.2</td>
</tr>
<tr>
<td>Diazaized</td>
<td>5</td>
<td>1.4</td>
<td>99.9</td>
</tr>
<tr>
<td>Hydrazide</td>
<td>100</td>
<td>40</td>
<td>22.9</td>
</tr>
<tr>
<td>Hydrazide + Protein A</td>
<td>100</td>
<td>21</td>
<td>36.8</td>
</tr>
<tr>
<td>Hydrazide + goat-anti-mouse Fc</td>
<td>100</td>
<td>9</td>
<td>67.1</td>
</tr>
<tr>
<td><strong>Polyamide beads</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Adsorption</td>
<td>5</td>
<td>2.9</td>
<td>67.9</td>
</tr>
<tr>
<td>Acid hydrolyzed + adsorption</td>
<td>5</td>
<td>4.0</td>
<td>76.9</td>
</tr>
<tr>
<td>Acid hydrolyzed + glutaraldehyde</td>
<td>5</td>
<td>3.7</td>
<td>66.3</td>
</tr>
<tr>
<td>Acid hydrolyzed + carbodiimide</td>
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<td>5.0</td>
<td>74.1</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>28.9</td>
<td>81.1</td>
</tr>
</tbody>
</table>

* A<sub>405</sub> after 2-h extraction at room temp. and 30-min incubation at 37 °C with CK reagent, expressed as percent of value obtained by using 2.5 μg of antibody per bead, passively adsorbed to polystyrene.

Analytical Variables

In this paper we use the term "total imprecision" to refer to assay-to-assay imprecision, which is a combination of the independent components of "within-assay imprecision" and "between-assay imprecision" (12, 13). Total assay imprecision of assaying the quality-control materials was calculated over a five-month period at Barnes Hospital and over a four-month period at Jewish Hospital. We estimated the components of within- and between-assay imprecision of quality-control material and serum samples, run in duplicate, from an analysis of variance for a hierarchical design with unequal replication (14).

Analytical-recovery studies were performed by adding 10 µL of HISP containing CK-MB—0 (control), 109, or 591 U/L—to 100 µL of patients' samples with endogenous CK-MB activities of approximately 10 U/L. The CK-MB activity was then measured in the direct assay. Analytical recovery was calculated by the following formula:

\[
[(\text{amount found} - \text{control amount})/\text{amount added}] \times 100
\]

We also assessed recovery by adding CK-MB to three lipemic samples with triglyceride concentrations of 16.6, 27.3, and 36.0 g/L to an icteric sample containing 0.52 g of bilirubin per liter, and also to an hemolytic containing 7 g of hemoglobin per liter.
covalent linkage led to immobilization of larger amounts of antibody, but the ability of the immobilized antibody to bind CK-MB was decreased, possibly owing to steric hindrance or inter- or intra-molecular cross-linking of the antibody molecules themselves.

The passive adsorption of antibody to polystyrene beads appeared essentially irreversible. More than 95% of $^{131}I$-labeled Concan-MB initially bound remained attached after the extraction and washing steps of the assay. In addition, we electrophoresed serum supplemented with CK-MB (110 U/L) and incubated with Concan-MB, either in solution or passively adsorbed to polystyrene beads. The serum exposed to the passively adsorbed Concan-MB showed no evidence of a CK-MB-antibody complex, although one was clearly visible when Concan-MB equal to 1% of that passively adsorbed was added in solution.

After determining the coating technique, we optimized the coating density. Although at least 6.5 μg of Concan-MB could be passively adsorbed to each bead, there was no increase in signal in the direct assay at amounts > 1.5 μg per bead (Figure 1). We now routinely coat the beads with 5 μg of antibody per bead; the resulting coating density is 2–3 μg per bead. Agitating the beads or recirculating the coating solution did not seem to increase the adsorption of antibody or improve the performance of the coated beads (data not shown).

In developing this assay, we initially used commercial CK reagent (Electro-Nucleonics) and monitored the NADH concentration at 340 nm. For the extraction step we used 60-well reaction trays (Abbott Laboratories; 12 × 30 × 2.5 cm, with 0.8-cm i.d. round-bottomed wells), which allowed use of available bead-washing equipment. One-hundred microliters of serum suffices to cover the beads. With constant shaking at room temperature, the extraction of CK-MB is essentially complete after 1 h (Figure 2). Approximately half of the CK-MB is extracted from serum under these conditions, and the extraction efficiency is constant over a wide range of CK-MB activities (data not shown).

In our previous assay we stopped the catalytic reaction at the end of the 37°C incubation by chilling the reaction mixtures and centrifuging to remove the latex particles. We have simplified this step by instead adding a solution that stops the enzymatic reaction (Figure 3) by chelating magnesium, which is required for CK enzymatic activity (2). The stopping solution contains Tris buffer, pH 7.5, and CDTA, which has an extremely high affinity for magnesium at this pH (17). The $A_{340}$ after the addition of CDTA at concentrations of 4 mmol/L or greater was 0.0014/min.

We developed a colored end product so we could use available colorimeters and could enhance the signal/noise ratio. The tetrazolium dye, INT, is reduced to its colored formazan in the presence of NADH and an electron carrier (18). The reduced form of INT has an absorptivity of 1940 L x mol$^{-1}$ x mm$^{-1}$ at 492 nm, which is approximately three-fold higher than that of NADH at 340 nm. We originally
tested phenazine methosulfate as the intermediate electron carrier, but found that the final absorances were not stable. We tested diaphorase as the electron carrier and found it more suitable. We optimized the concentrations of diaphorase and INT (Figure 4) and combined the stopping and color reagents in a single solution. The color development is complete within 2 min and the color is stable for several hours without removal of the beads (data not shown). The assay curve is linear to 200 U/L CK-MB (Figure 5) and the absorbance of the 12-U/L standard is threefold greater than background.

We have tested this system for feasibility in a kinetic assay by combining INT and diaphorase or phenazine methosulfate with the CK reagent in the enzymatic step. In these experiments there was very little color development over 30 min at 37 °C. We therefore utilized the reagents in two steps as described.

We prepared CK reagent without sulphydryl activator to prevent non-enzymatic reduction of INT (18). To ensure that CK-MB in serum samples was activated, we included 1 to 100 mmol of β-mercaptoethanol per liter in the extraction step of the assay. The measured CK-MB activities were identical at all concentrations of β-mercaptoethanol and equal to that found by using a commercial CK reagent containing sulphydryl activator. The same samples assayed without β-mercaptoethanol had substantially decreased CK-MB activity (data not shown). We therefore add 20 mmol of β-mercaptoethanol per liter to each sample.

Assay Performance

Nine 10 000-bead lots were prepared with an average coating density of 2.25 μg of Conan-MB per bead. We observed no difference in performance between any of the lots, and the coated beads could be used for at least eight months.

We assessed analytical recovery by supplementing serum samples (CK-MB values of 13.4, 8.7, or 10.3 U/L) with additional CK-MB and assaying them. Recovery in three assays, with added amounts of 10.9 and 59.1 U/L, averaged 111.9% and 107.4%, respectively. Recoveries of 45.4 U of added CK-MB per liter from lipemic samples with triglycerides of 16.6, 27.3, and 36.0 g/L were respectively 112.6, 108.4, and 110.8%. We could account for 110.4% from an icteric sample (0.52 g of bilirubin per liter) and 96.7% from an hemolysate (7 g of hemoglobin per liter).

We assessed interference in the direct colorimetric assay by measuring apparent CK-MB values in HISP containing CK-MB (26.3 U/L) with and without addition of CK-MM and/or CK-BB (Table 2). At the highest amounts of CK-MM added (92500 and 9250 U/L), we measured activities of 29.7 and 26.6 U/L, which were within the recovery limits found with CK-MB alone. At the highest values of CK-BB tested (82750 and 8275 U/L), 62.5 and 34.8 U/L were found. These activities are 33.0 and 5.3 U/L above those found with CK-MB alone, but represent less than 0.06% cross reactivity. Interestingly, when CK-MM and CK-BB were added simultaneously at these high concentrations, 101.7 and 40.1 U of apparent CK-MB activity per liter were detected. These increased activities were shown by electrophoresis to be CK-MB formed by hybridization of CK-MM and CK-BB and cannot be considered as cross reactivity.

The total imprecision over a five- or four-month period at Barnes or Jewish Hospitals was 14.1% and 16.8% for the lower concentration of quality-control material and 11.6% and 14.8% at the higher concentration (Table 3). We assessed the within-assay and between-assay contribution to the total assay variance and compared the imprecision of quality-control materials to that of patients' samples at Barnes Hospital. The larger component of imprecision was within-assay except for the patient group with CK-MB values >110 U/L, all of which were assayed after dilution. Dilution error could account for the higher between-assay component of imprecision of this group. The precision for patients' samples was found to be comparable to that for the quality-control materials.

Results of the direct routine assay compared well with those of a two-site immunoenzymometric mass assay (n = 226) (Figure 6). The linear regression equation was y (direct assay) = 0.800x (mass assay) + 0.69, with a correlation coefficient of 0.978. In addition, 413 samples had CK-MB activities <3 U/L and mass concentrations <3 μg/L. During

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**Table 2. Cross Reactivity of CK-MM and CK-BB in Measurement of CK-MB (U/L) by Direct Colorimetric Assay**

<table>
<thead>
<tr>
<th>U/L added*</th>
<th>CK-MM</th>
<th>CK-MB</th>
<th>CK-BB</th>
<th>U/L found*</th>
<th>% cross reactivity*</th>
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<tr>
<td>92500</td>
<td>26.3</td>
<td>—</td>
<td>29.5 ± 0.8</td>
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<td>29.5 ± 0.8</td>
<td>29.7</td>
<td>0</td>
</tr>
<tr>
<td>9225</td>
<td>26.3</td>
<td>—</td>
<td>29.5 ± 0.8</td>
<td>29.7</td>
<td>0</td>
</tr>
<tr>
<td>93</td>
<td>26.3</td>
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<td>29.5 ± 0.8</td>
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<tr>
<td>—</td>
<td>26.3</td>
<td>82750</td>
<td>62.5</td>
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</tr>
<tr>
<td>—</td>
<td>26.3</td>
<td>8275</td>
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<td>—</td>
<td>26.3</td>
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<td>0.10</td>
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<tr>
<td>—</td>
<td>26.3</td>
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<td>83</td>
<td>28.9</td>
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</tbody>
</table>

*U/L determined kinetically in the "Flexigem" centrifugal analyzer.

*Apparent CK-MB (U/L) activity measured in direct colorimetric assay.

[/(U/L found - U/L CK-MB) + U/L added] × 100.
a six-month period at Barnes Hospital, 347 samples with CK-MB values ≥12 U/L were checked by electrophoresis on agarose gel, which confirmed the increase in CK-MB in 339 cases. Of the samples showing positive results in the direct activity assay and negative results by electrophoresis, four had been assayed before clotting was complete. Upon reassay the CK-MB activity result agreed with electrophoresis. One sample showed a weakly positive signal on electrophoresis (estimated at ~8 U/L), approximately half of its result by direct assay (15 U/L). One sample was negative on electrophoresis and negative (6 U/L) on a repeat direct-activity assay after an initial determination of 14 U/L. Two other samples with values of 17 U/L and 47 U/L in the direct activity assay were negative for CK-MB by electrophoresis and were not repeated by either the direct assay or electrophoresis. For the patient with the value of 17 U/L, another sample obtained 7 h later gave a value of 11 U/L. Total CK was 978 and 791 U/L for the two specimens. The patient, admitted after an automobile accident, had a history of angina and hypertension and had a demand pacemaker. For the patient with the value of 47 U/L, previous CK-MB values were <4 U/L. The value of total CK for this sample was 256 U/L while those for previous samples were 33 to 59 U/L. Subsequent total CK values were 190, decreasing to 68 U/L two days later. CK-MB as measured by another laboratory was 6 U/L for a sample obtained 11 h after the sample that gave us the increased value. Lactate dehydrogenase isoenzyme 1:2 ratios were 0.4–0.7 before and after the increased CK-MB. The patient had major abdominal surgery two days before the elevated CK-MB value and had a history of coronary artery disease, confirmed by cardiac catheterization. She was transferred to the cardiac-care unit on the day the sample with increased CK was obtained, where she was treated for myocardial infarction. She had additional episodes of chest pain two days after the admission to the cardiac-care unit.

**Discussion**

CK-MB is measured in >25,000 samples per year at the clinical chemistry laboratories of Barnes and Jewish Hospitals. Most recently CK-MB had been quantified by use of Behring's Enzygnost™ assay kits. Some problems with this assay had developed (19) and the test was subsequently removed from the market. We evaluated several alternative commercial assays but failed to find a suitable substitute. We therefore modified a previously developed assay based on our monoclonal antibody to CK-MB (8) to make it suitable for routine laboratory use.

We believed that both precision and ease of handling could be improved by immobilization of Conan-MB onto larger beads than we had previously used. The availability of Frequantum and Pentawash II bead-washing systems led us to utilize 0.64-cm beads. Likewise, the available spectrophotometers required larger final volumes for measurement than our previous assay, and so we increased the signal by prolonging incubation times and using a colored product rather than direct measurement of NADH at 340 nm.

The total imprecision of the assay at the low quality-control value (10 U/L) was an improvement over that reported (18.9%–63.1%) for a pool with a similar concentration in evaluation of commercially available CK-MB assays (7). The direct assay was rigorously evaluated after several months of routine laboratory use by six or 14 medical technologists at Barnes and Jewish Hospitals, respectively, using multiple lots of reagent. The higher imprecision at

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**Table 3. Imprecision of the Direct Colorimetric Assay**

<table>
<thead>
<tr>
<th>Material assessed</th>
<th>Mean value, U/L</th>
<th>n</th>
<th>Total imprecision</th>
<th>Within-assay* component</th>
<th>Between-assay* component</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>SD CV, %</td>
<td></td>
<td>SD CV, %</td>
<td>SD CV, %</td>
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*Low quality-control and high quality-control materials were run in triplicate; patients' samples were run in duplicate.

*Samples from this group were assayed after dilution.

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**Fig. 6. Comparison of CK-MB activity (U/L) determined by direct colorimetric assay and CK-MB mass (μg/L) determined by two-site immunoenzymometric assay**

The linear regression equation (n = 226) was y = 0.800x + 0.69 (r = 0.978). CK-MB values of 413 additional samples were <3 by both assays.
Jewish Hospital is probably due to the lesser automation, particularly in the washing step, and the greater number of people performing the assay.

The accuracy of the assay was excellent as judged by the recovery, interference studies, and comparison with a two-site immunoassay and agarose gel electrophoresis. We found a small (0.06%) influence of CK-BB with this assay configuration that we did not observe with our previous assay format (8). We are not certain whether this small cross reaction is actual or is instead ascribable to hybridization of our CK-BB preparation with heat-inactivated CK-MM. We did observe a sizable amount of hybridization between this preparation of CK-BB and purified CK-MM. At any rate, even the 0.06% cross reactivity would not be a problem at the CK-BB concentrations that are present in serum.

In conclusion, our production and use of a monoclonal antibody specific for the MB isoenzyme of creatine kinase has allowed us to develop a simple, precise, and accurate assay for this important analyte. This assay has been in routine use at the chemistry laboratories at Barnes and Jewish Hospitals for eight months, with excellent results. The final form of the assay was dictated by the requirements of these two laboratories. However, the basic method is flexible and can be modified depending on the type of equipment available and sensitivity required. We expect that commercially available assays involving this antibody will soon be available.

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References