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Background: The AACC assembled a committee to identify and validate a standard creatine kinase MB isoenzyme (CK-MB) material to improve the comparability of CK-MB mass assays.

Methods: Three protocols were used. In protocol I, various CK-MB materials prepared in different matrices were screened as candidate standards. In protocol II, participating manufacturers calibrated their systems with concentrates of human heart CK-MB and then tested 20 patient samples to evaluate calibration bias. In protocol III, participating manufacturers calibrated their immunoassay systems using recombinant CK-MB2 (rCK-MB2) diluted into their respective sample diluents and measured 50 samples.

Results: Candidate materials showed high recovery in stripped human serum, but bias improved only from 59% to 38%. These data led to the use of human heart CK-MB diluted in each manufacturer's sample diluent.

Conclusion: Lyophilized rCK-MB2 was determined suitable for use as a reference material for CK-MB mass assays.

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Increased creatine kinase (CK;11 EC 2.7.3.2) isoenzyme MB (CK-MB) is considered the benchmark for diagnosis of acute myocardial infarction (AMI) (1, 2). A variety of methods are used to measure CK-MB, including electrophoresis, column chromatography, immunoinhibition/immunoprecipitation, and immunoassays (1, 3, 4). Additionally, assay methods such as immunoinhibition and electrophoresis rely on CK-MB activity (U/L). In recent years, many new CK-MB immunoassays have become available. These methods are termed “mass” assays because they measure CK-MB in terms of protein concentration, i.e., μg/L, using two-site immunoassay technology based on anti-M subunit (anti-CK-M), anti-B subunit

This strategy reduced bias from 31% to 15%. Because human heart CK-MB is difficult to provide, a lyophilized source of CK-MB2 was identified. rCK-MB2 was shown by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, reversed-phase HPLC, intrinsic protein fluorescence, circular dichroism, agarose gel electrophoresis, immunoreactivity studies, high and low temperature stability, and reconstituted stability to be equivalent to human heart CK-MB. Calibration of immunoassay systems with rCK-MB2 added into each respective manufacturer's sample diluent showed a 13% between-manufacturer bias.

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†Nonstandard abbreviations: CK, creatine kinase; CK-MB, CK-MM, and CK-BB, isoenzymes MB, MM, and BB of CK, respectively; AMI, acute myocardial infarction; CK-M and CK-B, M and B subunits of CK, respectively; rCK-MB2, recombinant CK-MB2 (tissue isoform); SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; and rCK-MB1, recombinant CK-MB1 (serum isoform, missing the C-terminal lysine on the M subunit).
or anti-MB-specific antibodies. CK-MB mass assays can be automated, are highly sensitive (detection limit, $1 \mu g/L$) and specific, and have a rapid turnaround time (as low as 7 min), giving them characteristics that surpass any other technology or measurement of CK-MB (4). The characteristics of different CK-MB mass assays utilized for the correlation studies are displayed in Table 1. Split patient sample correlation studies have shown that commercially available CK-MB immunoassays correlate well ($r > 0.98$) but are biased from one another, which is reflected by differences in the slopes of comparative analyses. When Henderson et al. (5) monitored the analytical performance of total CK ($\sim$230 participants) and CK-MB ($\sim$160 participants), they reported a wide dispersion of results between different analyzers as well as among identical analyzers. A major reason for such between-method bias is the lack of a CK-MB mass standard. To address this issue, the CK-MB Mass Assay Standardization Subcommittee was formed in January 1992 by the AACC to “identify or develop a standard material using human CK-MB that can be used to reduce laboratory-to-laboratory variation in the accuracy of the CK-MB mass assays”.

The goals of this committee were to identify and validate a source of CK-MB standard, to establish purification and protein determination protocols, and to investigate the effect that various matrices and CK-MB materials (liquid or lyophilized) have on standardizing CK-MB mass assays. With cooperation among manufacturers, it is hoped that this reference material will be used to standardize assay methods such that between-assay bias will be reduced or eliminated.

### Materials and Methods

**Expression and Purification of rCK-MB**

The cDNA sequences encoding CK-M and CK-B were cloned from a human cardiac cDNA library (6, 7). The cDNA for each subunit was subcloned into different *Escherichia coli* expression vectors, and the plasmids were cotransformed into *E. coli*. A strain was shown to express active CK-MB isoenzyme in vivo. Recombinant human CK-MB2 (rCK-MB2) was purified according to a modification of the method of Chen et al. (8). Purified protein was concentrated by diafiltration, and its concentration was determined by its absorbance at 280 nm. For use as a standard material, rCK-MB2 is formulated with stabilizers, including a carrier protein, dispensed into vials, and lyophilized.

**Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis Analysis**

Native heart CK-MB and rCK-MB2 were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to ascertain protein purity on 12% Tris-HCl ready gels (Bio-Rad). Gels were run on MiniProtein II systems (Bio-Rad) and stained with Coomassie blue.

**Agarose Gel Electrophoresis**

Agarose gel electrophoresis was used to verify CK isoenzyme purity via separation by overall protein charge. Native heart CK-MB and rCK-MB2 were applied to Universal Gel/8 agarose gels and electrophoresed according to the manufacturer’s protocol (Chiron Diagnostics). Gels were stained for CK activity using the Creatine Phosphokinase Isoenzymes kit (Sigma Diagnostics).

**Reversed-phase HPLC**

The purity and hydrophobic nature of native heart CK-MB and rCK-MB2 were analyzed by reversed-phase HPLC using triplicate 10-μg sample injections on a 2.1 × 30 mm Brownlee RP-300 (C$_3$) column on a Hewlett Packard 1090 HPLC system with photodiode array detection (Hewlett Packard). Proteins were eluted at a flow rate of 0.25 mL/min, using a linear gradient of 0.8 g/L trifluoroacetic acid in acetonitrile and detected at 214 nm. Hewlett Packard Chemstation software was used to integrate the data.

### Table 1. Characteristics of various CK-MB mass immunoassays.

<table>
<thead>
<tr>
<th>Analyzer</th>
<th>Capture antibody</th>
<th>Detection antibody</th>
<th>Label</th>
<th>Detection scheme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott AxSYM</td>
<td>Anti-CK-MB</td>
<td>Anti-CK-MM$^a$</td>
<td>Alkaline phosphatase</td>
<td>Fluorescence</td>
</tr>
<tr>
<td>Abbott IMx</td>
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<td>Anti-CK-MM$^a$</td>
<td>Alkaline phosphatase</td>
<td>Fluorescence</td>
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<tr>
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<td>Alkaline phosphatase</td>
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<tr>
<td>Beckman Access</td>
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<td>Anti-CK-MB</td>
<td>Alkaline phosphatase</td>
<td>Chemiluminescence</td>
</tr>
<tr>
<td>Dade Behring Opus Plus</td>
<td>Anti-CK-MB</td>
<td>Anti-CK-MM$^a$</td>
<td>Alkaline phosphatase</td>
<td>Fluorescence</td>
</tr>
<tr>
<td>Chiron ACS:180$^a$</td>
<td>Anti-CK-BB</td>
<td>Anti-CK-MB</td>
<td>Acidinium ester</td>
<td>Chemiluminescence</td>
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<tr>
<td>Dade Behring Stratus II</td>
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<td>Anti-CK-BB</td>
<td>Alkaline phosphatase</td>
<td>Fluorescence</td>
</tr>
<tr>
<td>Dade Behring aca Plus</td>
<td>Anti-CK-B</td>
<td>Anti-CK-MB</td>
<td>$\beta$-Galactosidase</td>
<td>Fluorescence</td>
</tr>
<tr>
<td>Hybritech Tandem-E$^b$</td>
<td>Anti-CK-B</td>
<td>Anti-CK-M</td>
<td>Alkaline phosphatase</td>
<td>Absorbance</td>
</tr>
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<td>Ortho Vitros:ECI</td>
<td>Anti-CK-BB</td>
<td>Anti-CK-MB</td>
<td>Horseradish peroxidase</td>
<td>Luminescence</td>
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<td>Roche Elecsys</td>
<td>Anti-CK-MB</td>
<td>Anti-CK-MB</td>
<td>Ruthenium complex</td>
<td>Chemiluminescence</td>
</tr>
<tr>
<td>TOSOH AIA 600</td>
<td>Anti-CK-BB</td>
<td>Anti-CK-MB</td>
<td>Alkaline phosphatase</td>
<td>Fluorescence</td>
</tr>
</tbody>
</table>

$^a$ Goat polyclonal antibodies. All other antibodies are murine monoclonal antibodies.

$^b$ Except for Chiron ACS:180, all anti-CK-MB antibodies are Conan antibodies.

$^c$ Tandem-E is no longer manufactured.
INTRINSIC PROTEIN FLUORESCENCE
The structural environments surrounding tryptophan residues in the proteins were assessed by measuring the intrinsic protein fluorescence of native heart CK-MB and rCK-MB2 at ambient temperature with a McPherson FL-750 spectrofluorometer (McPherson). Fluorescence excitation spectra were obtained by scanning the sample, 10 μmol/L CK-MB, from 200 to 300 nm at an emission maxima of 320 nm. The respective fluorescence emission spectra were measured by scanning from 300 to 400 nm at an excitation maxima of 290 nm. The relative fluorescence intensity data for both native heart CK-MB and rCK-MB2 were imported into a graphics program and displayed in overlapping plots.

CIRCULAR DICHOISM
The protein-folding conformation of native heart CK-MB and rCK-MB2 was analyzed by measuring their circular dichroism at 25 °C on a JASCO J720 spectropolarimeter (Jasco). A far-ultraviolet circular dichroism spectrum was obtained by scanning 0.5 g/L samples from 178 to 260 nm with a 0.005-cm pathlength.

IMMUNOREACTIVITY
The dose–response of native heart CK-MB and rCK-MB2 added to serum was assessed using an IMx immunoanalyzer. A 5 g/L working stock solution of CK-MB was diluted into heat-inactivated newborn calf serum (Genzyme Diagnostics) to 0, 3, 10, 30, 100, and 300 μg/L CK-MB. These samples were assayed in duplicate in separate IMx analytical runs using the same stored calibration curve. Their fluorescence reaction rates were plotted against standard dilution factors. The native heart CK-MB and rCK-MB2 response curves in the immunoassay were overlayed for comparison.

ACCELERATED STABILITY STUDIES of rCK-MB2
To assess the storage shelf-life of lyophilized rCK-MB2 at 2–8 °C, accelerated stability studies were conducted by stressing vials of rCK-MB2 in isothermal incubators set at 45, 37, and 30 °C at staggered time intervals. For each temperature, stressed vials were reconstituted at the same time with Genzyme Diluent (Genzyme Diagnostics), diluted into assay range with heat-inactivated newborn calf serum (Genzyme Diagnostics), and assayed in duplicate in the same analytical run, using the IMx CK-MB mass assay. The percentages of recovery were determined relative to that of the value for non-stressed CK-MB stored at 2–8 °C.

The rate constant for the loss of CK-MB mass at each incubation temperature was determined by linear regression analysis of the time plot data using a graphical program. An Arrhenius plot was constructed using the logarithm of the rate constants against the inverse of incubation temperature in degrees Kelvin. From the Arrhenius plot, the rate constant for the loss of rCK-MB mass at 8 °C can be extrapolated by linear regression analysis and used to calculate the time for rCK-MB mass to decrease 10% at 8 °C (9).

RECONSTITUTION STABILITY of rCK-MB2
The reconstituted or opened vial stability for rCK-MB2 was performed by reconstituting vials with Genzyme CK-MB Diluent (Genzyme Diagnostics), storing them at 2–8 °C, and removing aliquots from these vials at different time intervals for assay in duplicate, using an IMx immunoanalyzer. The reconstituted product stability of rCK-MB2 at 2–8 °C was measured by the decrease in CK-MB mass to 90% of its day 0 value.

SHELF-LIFE STUDY
Lyophilized rCK-MB2 was stored at 4 and −20 °C. At each selected time point over a period of ~500 days, rCK-MB2 was reconstituted in acetyl sample diluent, and its mass was measured using the acetyl Plus immunoanalyzer. A 10% loss of CK-MB mass in vials stored at 4 °C relative to that stored at −20 °C was used as the stability criterion.

Results
The standardization process followed in this study was divided into four phases. It is of note that all CK-MB immunoassays were compared to IMx because of its availability in all of the participating laboratories and not because it was considered the reference method.

PHASE 1: SCREENING OF CK-MB CANDIDATES
CK-MB candidates. Four different forms of CK-MB were considered for the development of a CK-MB standard: human heart CK-MB, hybrid CK-MB, rCK-MB2 (tissue isoform), and rCK-MB1 (serum isoform, missing the C-terminal lysine on the M subunit). The hybrid CK-MB, prepared by reassociation of the respective M and B subunits of denatured CK-MM and CK-BB, was obtained from Aalto Scientific. Different recombinant CK-MB forms were provided by Dr. M. Benjamin Perryman (University of Colorado Health Science Center, Denver, CO) (10). In addition, during the later stage of evaluation (phase 3), lyophilized rCK-MB2 was provided by Genzyme Diagnostics.

Purification of CK-MB. Human heart CK-MB, rCK-MB2, and rCK-MB1 were purified using anti-CK-MB monoclonal antibody immunoaffinity chromatography according to established procedures (11). The final preparations of CK-MB were stored at −20 °C in 20 mmol/L Tris-HCl, pH 7.2 containing 500 mL/L glycerol. The purity of all CK-MB preparations was assessed by SDS-PAGE and agarose gel electrophoresis.

Determination of CK-MB concentration. From the protein sequence of CK-MB, one can determine that the B subunit of CK contains 4 tryptophan, 9 tyrosine, and 2 cystine.
amino acid residues, whereas the M subunit of CK contains 4 tryptophan, 10 tyrosine, and 2 cystine amino acid residues (6, 10). Based on this amino acid content, the molar absorptivity of CK-MB was estimated as 0.82 L/cm-mol (absorbance of 1 g/L solution of CK-MB at 280 nm using 1-cm pathlength) (12).

The concentration of the purified CK-MB was determined spectrophotometrically on a Model 8450A Spectrophotometer (Hewlett Packard).

Lyophilized and liquid forms of human heart native CK-MB, rCK-MB1, rCK-MB2, and hybrid CK-MB were evaluated in different matrices, which yielded equivalent recovery results in immunoassays. Lyophilized rCK-MB2 was selected for use in all subsequent studies because of its long-term stability and ease of shipping and handling.

**PHASE 2: DETERMINATION OF OPTIMUM CalIBRATOR MATRIX**

**Matrix selection.** Three different potential sample matrices were evaluated: an unprocessed CK-MB-free human serum pool, stripped serum, and a synthetic matrix (50 mmol/L phosphate buffer containing 50 g/L bovine...
serum albumin and 0.15 mol/L NaCl, pH 7.4). The stripped human serum was prepared by mixing a human serum pool with a blend of anion- plus cation-exchange resin to extract residual CK. Some other serum components may have been removed unintentionally during this process. The stripped human serum was donated by Dade Behring (Glasgow, DE). Manufacturers’ sample diluents were subsequently evaluated as the study progressed.

**Formulation.** To optimize the formulation of a standard for use in all CK-MB mass assays, a screening experiment was designed in which the four sources of CK-MB and the three matrices were evaluated. In this study, formulations containing 0, 10, or 100 µg/L of human heart CK-MB, hybrid CK-MB, rCK-MB1, or rCK-MB2 were prepared in three matrices. These samples were shipped to nine participating manufacturers of CK-MB mass assays. At each manufacturer’s site, each vial was hydrated with 2 mL of deionized water, and the CK-MB masses of the reconstituted candidate standards were determined on their respective immunoassay systems.

**Manufacturers’ correlation study: protocol I.** Purified, lyophilized human heart CK-MB candidate standards (0, 10, and 100 µg/L) prepared in stripped human serum were assayed on each immunoassay analyzer and later used to correct original calibration curves via regression analysis. Additionally, 69 patient samples were analyzed for...
CK-MB mass. For these studies, the following seven immunoassay systems were used: ACS:180 (Chiron Diagnostics), Stratus II (Dade-Behring Diagnostics; analysis performed at the Glasgow, DE site), Tandem-E (Hybritech), OPUS Plus (Dade Behring; analysis performed at the Glasgow, DE site), accu Plus (Dade Behring; analysis performed at the Wilmington, DE site), AIA-600 (TOSOH), and IMx (Abbott Diagnostics). Assays were conducted according to respective manufacturers’ specifications.

Manufacturers’ correlation study: protocol II. In an alternative approach for the standardization of CK-MB mass assays, the manufacturers of these assays were provided 20 patient sample pools (not used in the study described above) and purified human heart CK-MB from which each participating manufacturer prepared CK-MB candidate standards at 0, 10, 50, 100, and 200 µg/L in their sample diluent. The standards and the samples were analyzed in duplicate in their respective immunoassay systems. The slope bias of these methods against IMx was determined by linear regression analysis.

For the different calibrator matrices, CK-MB recovery (data not shown) varied over a wide range as follows: stripped human serum (60–100%), human serum (23–60%), and synthetic medium (29–88%). Although stripped human serum produced the highest CK-MB recovery, the utilization of this matrix for reconstitution of CK-MB reference material only slightly reduced the slope range from ±59% to ±38% (Fig. 1). The slight reduction in between-manufacturer bias achieved with candidate standards made with stripped human serum is probably attributable to the variable effect of this matrix in different immunoassays. After dilution of human heart CK-MB reference material using the manufacturers, sample diluents, the range of slopes for the patient correlation plots was reduced from ±31% to ±15% (Fig. 2).

Because all CK-MB isoforms were recovered similarly in different matrices and native human CK-MB preparations are difficult to obtain and prone to batch-to-batch variability, rCK-MB2 is proposed as a reference material.

**Phase 3: Preparation and properties of rCK-MB2**

After phases 1 and 2 were completed, lyophilized rCK-MB2 was evaluated as a CK-MB reference standard. To validate its similarity to native human heart CK-MB, this material was analyzed in structural, immunological, and stability studies and compared to native CK-MB obtained from either BioProcessing, Inc., or provided by Dr. Jack Ladenson (Washington University, St. Louis, MO) (11).

rCK-MB2 was expressed and purified from *E. coli*. The purified rCK-MB2 has an apparent molecular mass similar to native heart CK-MB, as shown by SDS-PAGE analysis (Fig. 3). The purity and structural similarity of rCK-MB2 to native heart CK-MB was further demonstrated by agarose gel electrophoresis (Fig. 4) and reversed-phase HPLC (Fig. 5) analyses.

The intrinsic protein fluorescence spectra for native heart CK-MB and rCK-MB2 are similar (Fig. 6). Circular dichroism studies of the native and recombinant CK-MB proteins revealed no distinguishable secondary structure (data not shown).

The immunoreactivity responses of native heart CK-MB and rCK-MB2 were examined using the IMx CK-MB mass assay, which showed that both proteins have similar immunoreactivity (Fig. 7).

Different studies were performed to evaluate the stability of rCK-MB2. In accelerated stability studies (Fig. 8), the percentages of recovery were determined relative to the value for CK-MB stored at 2–8 °C. The rate constant for the loss of rCK-MB mass at 8 °C was extrapolated by linear regression analysis from the Arrhenius plot and used to calculate the time for rCK-MB mass to decrease 10% at 8 °C. Studies of lyophilized rCK-MB2 showed that the refrigerated product shelf-life was 19 years (Fig. 9). The reconstituted product stability of rCK-MB2 at 2–8 °C was at least 21 days as measured by the decrease in CK-MB mass to 90% of its day 0 value (Fig. 10). The shelf-life of rCK-MB2 at 4 °C, as determined relative to its mass at −20 °C, was 555 days (data not shown).

**Phase 4: Final rCK-MB2 evaluation**

Each of the participating manufacturers was provided lyophilized rCK-MB2 and its diluent and 50 patient sample pools. Manufacturers reconstituted the rCK-MB2,
prepared rCK-MB2 candidate standards at 0, 10, 50, 100, and 200 μg/L in their respective sample diluents, and determined the CK-MB mass values of the rCK-MB2 candidate standards and 50 samples. Data were analyzed for between-manufacturer bias by assessing patient sample correlation plots.

A final correlation study was performed to evaluate the lyophilized rCK-MB2 on different CK-MB mass assays. As shown in Fig. 11, before using rCK-MB2 to establish the calibration curves for different CK-MB immunoassays, the range of slopes was ± 40%. This slope range was reduced to ± 13% after rCK-MB2 was used to establish the calibration curves for the immunoassays.

**Discussion**

This study showed that a lyophilized rCK-MB material is suitable for use as a reference material for standardization of CK-MB mass assays. This is important for reducing method-related bias of CK-MB mass assays.

Initially, the catalytic activity of CK in serum or plasma was measured to evaluate the diagnosis of AMI and to rule out cardiac necrosis. Recently, Gella et al. (13) described the preparation of lyophilized CK-MB purified from human heart for use as a reference material for CK-MB catalytic concentration measurements. Upon reconstitution, the catalytic concentration of the reference CK-MB material was reported to be ~67 U/L when measured at 30 °C by the recommended method of the International Federation of Clinical Chemistry (13).

CK-MB mass assays were developed to improve both the analytical and clinical sensitivity and specificity for measuring CK-MB compared with the enzymatic assay. These mass assays utilize monoclonal anti-CK-MB antibody in conjunction with anti-M or anti-B antibodies to accurately measure small changes in CK-MB concentrations during the early hours following AMI (Table 1).
Despite the fact that CK-MB expression is not limited to cardiac tissue and that its diagnostic accuracy is low (~50%) at 3 h after AMI, CK-MB is still regarded as the benchmark for diagnosis of AMI. In the Chest Pain Evaluation Center environment, Gibler et al. (14) examined the utility of assaying CK-MB mass in a strategy that included sampling at presentation and subsequently after 3, 6, and 9 h in 1000 low-risk nondiagnostic electrocardiogram patients. Although these patients were at low myocardial infarction risk, this study documented a sensitivity of 100% and specificity of 98.3% for myocardial infarction diagnosis in this population of chest pain patients (14).

The field of biochemical markers of cardiac injury is in a dynamic state, in which assays for novel markers and current markers such as cardiac troponin I and cardiac troponin T continue to be developed to enhance the diagnosis of AMI (15). However, progress in the development of several different biochemical assays for new AMI markers has led to several analytical and interpretative issues, particularly those pertaining to comparisons with different CK-MB immunoassays. Therefore, it was deemed essential by the AACC to establish rigorous criteria for standardizing CK-MB mass assays. To accomplish this task, the AACC established a subcommittee to identify and develop a standard CK-MB material to reduce method-related variations in the analytic accuracy of CK-MB mass assays.

The CK-MB standardization process was conducted in four phases: 

- Identification of a CK-MB candidate reference material,
- Determination of an optimum assay matrix,
- Validation of the biochemical characteristics of the identified reference material, and
- Testing the selected material on different CK-MB mass assays.

Although four different forms of CK-MB were examined, the results indicate that rCK-MB2 is the most suitable as a reference material because it can be easily

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**Table 1**

<table>
<thead>
<tr>
<th>Assay Brand</th>
<th>Reference Material</th>
<th>Specificity</th>
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<tbody>
<tr>
<td>Hybritech Tandem-E</td>
<td>rCK-MB2</td>
<td>100%</td>
</tr>
<tr>
<td>Chiron ACS:180</td>
<td>rCK-MB2</td>
<td>98.3%</td>
</tr>
</tbody>
</table>

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- Identification of a CK-MB candidate reference material,
- Determination of an optimum assay matrix,
- Validation of the biochemical characteristics of the identified reference material, and
- Testing the selected material on different CK-MB mass assays.

Although four different forms of CK-MB were examined, the results indicate that rCK-MB2 is the most suitable as a reference material because it can be easily
prepared in large, homogeneous quantities compared with the other CK-MB forms. Lyophilized rCK-MB2 was favored over that in a liquid format because of its stability and ease of handling. Furthermore, validation experiments in this study have shown that rCK-MB2 has physicochemical characteristics similar to, if not equivalent to, CK-MB purified from human heart (11).

Examination of three different assay matrices revealed that stripped human serum showed the best recovery. However, utilization of this matrix to reconstitute CK-MB material did not produce a significant reduction in method bias, which may be related to different manufacturers’ assay response to this matrix. Further experiments demonstrated that the use of each respective man-
manufacturer’s sample diluent to reconstitute a CK-MB reference material substantially reduced method bias to ± 15% (Fig. 2).

The findings of this study show that lyophilized rCK-MB2 can be used as a reference material for standardizing CK-MB mass assays. The systematic bias between the CK-MB methods was reduced from ± 40% to ± 13% with the use of rCK-MB2. Manufacturers should use this material to adjust their CK-MB mass assay calibration curves so that method-related bias is minimized or eliminated for this important AMI diagnostic tool.

The next steps of this process include (a) production and purification of large quantities of rCK-MB2, and (b) performance of studies necessary to gain acceptance of lyophilized rCK-MB2 as a worldwide CK-MB standard. Finally, this study may serve as a model for other future standardization activities of new cardiac markers, such as troponin I and myoglobin. In particular, standardization of biochemical markers of cardiac injury will be essential to achieve cost-effectiveness of diagnostic testing of AMI.

We thank Marcella Holdridge (Genzyme Diagnostics) for technical assistance. We thank the participants from various diagnostic immunoassay manufacturers for collecting data for the patient sample correlation studies. Finally, we acknowledge the diligence of Jean Rhame in working with the AACC CK-MB Mass Assay Standardization Subcommittee and in assisting with the assembly of this article.

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