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Assay of Creatine Kinase Isoenzyme MB in Serum with Time-Resolved Immunofluorometry

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We describe the first time-resolved immunofluorometric assay for creatine kinase (EC 2.7.3.2) isoenzyme MB (CK-MB) in serum. The assay is based on the formation of the complex: solid-phase anti-CK-MB–CK-MB–biotinylated anti-CK-BB–streptavidin–BCPDA–Eu^{3+}, where anti-CK-MB and anti-CK-BB are monoclonal antibodies against the CK isoenzymes MB and BB, respectively, and BCPDA is the europium chelator 4,7-bis(chlorosulfophenyl)-1,10-phenanthroline-2,9-dicarboxylic acid. The solid-phase complex is fluorescent and is measured on the dry solid-phase (microtiter well) in a specially designed time-resolved fluorometer that uses laser excitation. The assay requires 25 μL of serum and is not affected by the presence of either CK-MM (up to 5000 μg/L) or CK-BB (up to 1000 μg/L) in the sample. Precision and accuracy indices for the assay were satisfactory.

Additional Keyphrases: *acute myocardial infarction · biotin–streptavidin · europium chelates*

Measurement of serum creatine kinase (ATP:creatine *N*-phosphotransferase, EC 2.7.3.2) MB isoenzyme (CK-MB) (*1*) is still the most specific and sensitive indicator of myocardial necrosis and the most reliable and critical laboratory test for the biochemical diagnosis of acute myocardial infarction (AMI) (*2, 3*).[5] Thus, the specificity and sensitivity of laboratory tests for the determination of CK-MB are of critical importance (*4*).

Several methods for determining CK-MB in serum have been developed. Methods based on determining catalytic activity after separation of CK-MB from the CK-BB and CK-MM isoenzymes by electrophoresis or ion-exchange chromatography are generally semiquantitative and labor intensive (*5*). Immunological methods for CK-MB based on immunoinhibition or immunoprecipitation of CK-MM and measurement of catalytic activity of CK-MB are susceptible to interferences from CK-BB isoenzyme and macro-CK type 1 or mitochondrial CK (macro-CK type 2) (*6, 7*).

Recently developed two-site immunoassays for CK-MB are superior to other immunochemical methods in sensitivity and specificity. These "sandwich"-type assay configurations use highly specific anti-CK-MM, anti-CK-BB, or anti-CK-MB monoclonal antibodies. Several highly specific two-site immunoassays for CK-MB have recently become commercially available, e.g., the Stratus two-site fluorometric radial partition immunoassay (Baxter Healthcare Corp., Miami, FL 33152) (*8*), the MagicLite chemiluminometric immunoassay (Ciba Corning Diagnostics Corp., E. Walpole, MA 02032) (*9*), the Icon QSR assay (Hybritech, San Diego, CA 92126) (*10*), and the IMx fluorometric assay (Abbott Diagnostics, Abbott Park, IL 60064). Newly developed assays for CK isoforms in serum purportedly are even more specific markers for AMI but are not yet widely used (*11*).

Among the possible problems of the immunometric-type CK-MB assays are the falsely positive interferences by heterophilic antibodies (e.g., anti-mouse IgG antibodies present in human sera), which have been reported for many two-site immunoassays that involve mouse monoclonal antibodies (*12*). False-positive results were also reported for the Stratus CK-MB assay because of the presence of a high-molecular-mass form of alkaline phosphatase (*13*), although this interference seems to have been eliminated (*13*).

Recently, highly sensitive two-site immunoassays based on time-resolved fluorescence measurements of europium have been developed (*14*). Here, we describe the first time-resolved immunofluorometric assay of CK-MB in serum. The assay is based on the formation of an immunocomplex involving CK-MB, a specific monoclonal antibody to CK-MB immobilized on a solid phase (microtiter well), and a biotinylated monoclonal antibody specific for the B subunit. The formed immunocomplex is quantified by adding a highly sensitive 4,7-bis(chlorosulfophenyl)-1,10-phenanthroline-2,9-dicarboxylic acid (BCPDA)-labeled streptavidin-based macromolecular complex, which has been recently described (*15*), and by measuring the specific Eu^{3+} fluorescence in a time-resolved fluorometer.

Materials and Methods

Instrumentation

For time-resolved solid-phase fluorescence measurements we used the CyberFluor 615™ Time-Resolved Fluorometer/Immunoanalyzer (CyberFluor Inc., Toronto, Canada) with an excitation wavelength of 337.1 nm (nitrogen laser source) and an emission wavelength of 615 (±5) nm (interference filter). Specially designed software provides automated data reduction. White opaque microtitration wells (assembled in 12 wells per strip, Immulon II) were products of Dynatech Labs, Alexandria, VA 22314.

Materials

The europium chelator, BCPDA, was synthesized as previously described (*16*). Streptavidin and bovine serum albumin (BSA) were purchased from Sigma Chemical Co., St. Louis, MO 63178. Sulfosuccinimidyl 6-(biotinamido) hexanoate (NHS-LC-biotin) was from Pierce Chemical Co., Rockford, IL 61105.

Creatine kinase isoenzymes. CK-MB from human heart tissue (purity ≥99%), CK-MM from human skeletal muscle (purity ≥98%), and CK-BB from human brain (purity

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[5] Nonstandard abbreviations: CK, creatine kinase; CK-MB, CK-BB, and CK-MM, isoenzymes of CK; AMI, acute myocardial infarction; BCPDA, 4,7-bis(chlorosulfophenyl)-1,10-phenanthroline-2,9-dicarboxylic acid; SA, streptavidin; TG, bovine thyroglobulin; BSA, bovine serum albumin; and NHS-LC-biotin, sulfosuccinimidyl 6-(biotinamido) hexanoate.

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≥95%) were purchased from Scripps Laboratories, San Diego, CA 92131. All purities were established by electrophoresis. All other chemicals were from Sigma Chemical Co.

CK-MB standards. CK-MB standards at concentrations of 0–100 µg/L were prepared by appropriate dilutions of CK-MB in heat-inactivated and filtered normal pooled human serum. These standards were calibrated with the Stratus CK-MB assay and were kept at 4 °C when not in use and at −20 °C and for longer storage (>1 week).

Serum samples. Serum samples, obtained from 66 patients admitted to the coronary care unit of Toronto Western Hospital, were found positive for CK-MB by a screening immunoinhibition method (see below). Fifty-seven samples were obtained from a mixed population (Caucasian men and women) of apparently healthy subjects (blood donors), ages 20–40 years, for estimating the reference range.

Monoclonal antibodies. We purchased monoclonal antibodies for the B subunit of CK, anti-CK-BB (clone nos. 027-11288, 027-11612, 027-15712, 027-18812, 027-11267, 027-18432), as well as for the M subunit of CK, anti-CK-MM (clone nos. 027-17186, 027-18367, 027-12859, 027-11310, 027-14110), from OEM Concepts Inc., Toms River, NJ 08755. We have also purchased a specific monoclonal antibody for CK-MB, anti-CK-MB (lot no. 709107), and a specific monoclonal antibody for the B subunit of CK (lot no. 734916) from International Immunoassay Laboratories, Inc., Santa Clara, CA 95054. All monoclonal antibodies used in this study were purified from ascites fluid with an AffiGel protein A "MAPS" kit (Bio-Rad Laboratories, Richmond, CA 94804).

Procedures

Preparation of biotinylated antibodies. Monoclonal antibodies used as detection antibodies were biotinylated with sulfosuccinimidyl 6-(biotinamido) hexanoate (NHS-LC-biotin) as previously described (*17*). Working solutions of the biotinylated antibodies were prepared by diluting them to ~2.5–5 mg/L in 50 mmol/L Tris buffer, pH 7.40, containing 9 g of NaCl, 5 g of BSA, 0.5 g of NaN_3, 5 mL of normal mouse serum, 0.5 g of bovine γ-globulins, 0.5 mol of KCl, and 0.02 mL of Tween 40 per liter.

Preparation of labeled streptavidin. A streptavidin-based macromolecular complex, a very sensitive detection reagent, was formed by conjugating streptavidin with BCPDA-labeled thyroglobulin, as described elsewhere (*18*), and incubating the reaction mixture with Eu^{3+} as previously described (*15*). The concentration of streptavidin in this stock solution was 15 mg/L. Labeled streptavidin working solution was prepared by diluting the stock solution 50-fold in a 50 mmol/L Tris buffer, pH 7.20, containing, per liter, 40 g of bovine serum albumin, 9 g of NaCl, 0.1 g of sodium azide, and 40 µmol of Eu^{3+}.

Coating of microtitration wells. The wells were coated overnight at room temperature with the coating solution (50 µL/well), a 50 mmol/L Tris solution, pH 7.40, containing 10 µg of coating antibody per milliliter. After coating, the wells were washed with a wash solution containing 9 g of NaCl, 0.5 mL of polyoxyethylene sorbitan monolaurate (Tween 20), and 0.5 g of NaN_3 per liter, and blocked for 30 min at room temperature with 200 µL per well of a 50 mmol/L Tris solution, pH 7.40, containing 60 g of BSA and 0.5 g of sodium azide per liter. The wells were stored in the blocking solution and were washed just before use as described above.

Comparison methods. We used four different methodologies for comparison and other studies. As a screening procedure, we used the immunoinhibition method, with reagents from Boehringer Mannheim Diagnostics, Indianapolis, IN 46290, and a chemistry analyzer (Hitachi Model 717; Boehringer Mannheim). Sera found positive by this method were further analyzed by three different two-site sandwich immunoassays specific for CK-MB, i.e., the Stratus, the Icon QSR, and the IMx CK-MB assays, according to the manufacturers' recommendations.

Assay procedure. Wash coated and blocked wells twice with the wash solution before the assay. Add 50 µL of the working biotinylated antibody solution (2.5 mg/L) to each well, and pipet 25 µL of standards or serum samples (in duplicates) into each well. After shaking the plates for 30 min (or 15 min for the rapid procedure) at room temperature, wash the wells once with the wash solution, then add 50 µL of the labeled streptavidin working solution to each well. After 30-min incubation (or 15 min for the rapid procedure), wash the wells and then dry them with a forced-air plate dryer for 5 min at room temperature. The fluorescence of Eu^{3+} on the solid phase is measured with the CyberFluor immunoanalyzer. Data reduction is automatic.

Results

Selection of monoclonal antibodies. Thirteen monoclonal antibodies were tested in various combinations in an initial effort to establish workable pairs. In all cases, we used a solid-phase antibody (immobilized on microtiter wells) and a biotinylated detection antibody. For coating, we tested the seven anti-CK-BB antibodies in combination with five anti-CK-MM detection antibodies (35 different pairs) and an anti-CK-MB detection antibody (seven pairs). Additionally, we tested the anti-CK-MB antibody for coating and used either the anti-CK-BB (seven) or the anti-CK-MM antibodies for detection (total of 12 pairs). From the total 54 pairs tested, 24 pairs gave calibration curves. Further studies on cross-reactivity showed that only the pair of anti-CK-MB (coating antibody, lot no. 709107):anti-CK-BB (detection antibody, lot no. 734916) was free from both positive and negative interference by CK-MM (up to 5000 µg/L) or CK-BB (up to 1000 µg/L). To assess positive interference, we measured samples containing only CK-MM or CK-BB; we assessed negative interference by checking the percent reduction in the values found for a CK-MB sample initially measured as 20 µg/L in the presence of

Table 1. Interference Studies for the Proposed CK-MB Assay (Protocol A)

Detection antibody, ng/well	Positive interference[a]		Negative interference[b]	
	CK-MM	CK-BB	CK-MM	CK-BB
500	ND (0)[c]	0.10 (0.01)	21.9 (109)	21.9 (109)
250	ND (0)	0.26 (0.03)	19.7 (98.5)	21.6 (108)
125	ND (0)	0.35 (0.04)	20.1 (100)	22.4 (112)

[a] Expressed as µg/L of CK-MB; CK-MM and CK-BB were added to CK-MB-free serum at concentrations of 5000 and 1000 µg/L, respectively.

[b] Values shown are µg/L of CK-BB measured for a sample originally containing 20 µg of CK-MB per liter and then supplemented with CK-MM and CK-BB at concentrations of 5000 and 1000 µg/L, respectively.

[c] Numbers in parentheses represent % cross-reactivity (positive interference studies) or % of expected values (100% when negative interference is zero).

ND, not detected.

CK-MM or CK-BB (Table 1). Thus, we finally selected anti-CK-MB as the solid-phase capture antibody and anti-CK-BB (lot no. 734916) as the biotinylated detection antibody for the development of this assay.

Assay optimization. The coating of wells was optimized for antibody concentration. An antibody concentration of 500 ng/50 μL per well was chosen for maximum signal. We found that coating with 250, 125, or 60 ng per well yielded a signal of about 70%, 50%, and <40% of maximum, respectively.

With use of 500 ng per well of coating antibody, the assay was optimized for biotinylated-antibody concentration. An antibody concentration of 2.5 mg/L (125 ng/50 μL per well) was chosen as optimum because it gave the lowest background signal, even though additional antibody (250 and 500 ng per well) gave practically the same fluorescence signals but a higher background. To study the effect of incubation time on the first step, we incubated the samples in the coated wells with biotinylated antibody for 15, 30, 60, and 120 min, but kept the streptavidin-binding incubation step constant at 30 min. Fluorescence signals were increased by increasing the incubation time up to 120 min but with no significant increase in assay detectability because nonspecific binding (zero standard signal) also increased. The same studies were performed with the streptavidin incubation step.

On the basis of these studies, we devised two assay protocols: a 15 min–15 min rapid protocol (A) and a 30 min–30 min normal protocol (B); the times refer to the first and second incubation steps, respectively. To minimize errors caused by variable incubation times between wells (delay effect), we first pipetted the detection antibody in the wells and then we pipetted all standards and patients' samples within 10 min, for both protocols. We also added 5 mL of mouse serum per liter of antibody diluent to avoid any possible falsely increased results for CK-MB, owing to rheumatoid-like factors and anti-mouse immunoglobulin antibodies in some human sera (*12*).

Assay characteristics. Typical standard curves for both assay protocols are shown in Figure 1. Both curves are linear at concentrations of CK-MB ≤100 μg/L. The detection limit, defined as the analyte concentration corresponding to the fluorescence reading of the zero standard +2 SD, was 0.46 and 0.36 μg/L for the rapid and the normal protocol, respectively. The detection limit of the normal protocol showed no significant improvement because increasing the incubation times increased both the background and specific signal proportionally.

Precision. Within-run precision was determined by analyzing serum samples at three different concentrations, 12 times each, by both protocols. Day-to-day precision was assessed by analyzing another three serum samples 10 times over a period of 10 days (Table 2).

Dilution test. We evaluated the linearity of the method by diluting five different serum samples of known CK-MB concentrations (20–30 μg/L) with heat-inactivated normal serum (already found negative for CK-MB by the Stratus method) and by reassaying them by both protocols. For all cases, linear dilution curves at dilution factors ranged from three- to 20-fold.

Analytical recovery. Serum samples supplemented with CK-MB at two different concentrations were prepared by adding known concentrations of exogenous CK-MB to four pooled serum samples with CK-MB values between 0.5 and 1.1 μg/L. To assess analytical recovery, we analyzed the samples before and after the two additions of CK-MB (~18 and 40 μg/L), with mean analytical recoveries of 82.8% ±5.7% and 91.9% ±4.6% for protocols A and B, respectively.

Reference interval. We analyzed 57 serum samples from apparently healthy blood donors, ages 20–40 years, by both proposed protocols. The reference interval, defined as the range of values between the 2.5th and 97.5th percentile, was 0–4.1 μg/L. Measured values ranged from undetectable to 6.0 μg/L.

Correlation with other methods. We compared the results from the proposed time-resolved immunofluorometric assay for CK-MB with those obtained by three commercially available immunoassays for CK-MB in serum, i.e., the Stratus, Icon QSR, and IMx CK-MB (Table 3). The proposed assay correlates well with the Stratus (r = 0.978) as well as with the Icon (r = 0.987) and IMx (r = 0.984). However, the slope of the regression equation is close to unity (0.84–0.90) only with the Stratus system and is significantly lower (0.58–0.71) for the Icon and IMx methods. This is expected in that the Icon assay reportedly gives higher values than the Stratus (*19, 20*). Although the IMx assay (*21*) has not been widely used, it seems to give higher readings than the Stratus assay.

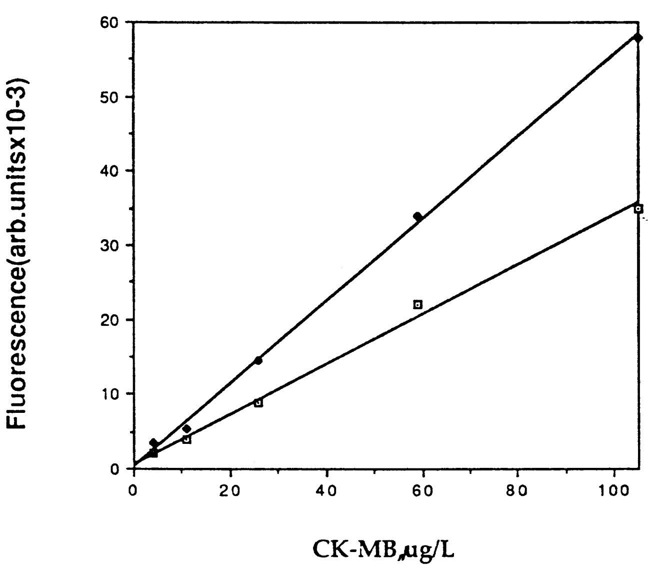

Fig. 1. Calibration curves for the proposed CK-MB assay for both protocols

□, rapid protocol (30 min); ◆, normal protocol (60 min). The fluorescence of the zero standard has been subtracted from all other fluorescence readings

Table 2. Precision of the CK-MB Assay

Sample	Protocol A: Mean (SD) CK-MB, μg/L	Protocol A: CV, %	Protocol B: Mean (SD) CK-MB, μg/L	Protocol B: CV, %
Within-run (n = 12)				
1	7.4 (0.4)	5.4	8.4 (0.5)	6.0
2	16.2 (1.0)	6.2	18.9 (1.1)	5.8
3	39.6 (1.6)	4.0	45.2 (1.9)	4.2
Day-to-day (n = 10)				
1	8.8 (1.1)	12.3	8.2 (0.9)	11.0
2	26.0 (1.9)	7.3	22.8 (1.8)	7.9
3	48.5 (4.0)	8.2	46.6 (4.6)	9.9

Table 3. Comparison of the Proposed CK-MB Assay with Three Two-Site Immunoassays for CK-MB

Comparison method (x)	Proposed method (y), protocol	CK-MB range, µg/L	Slope[a]	Intercept, µg/L	r	$S_{y,x}$
Stratus, n = 66	A	1.4–370	0.90	−0.29	0.978	17.0
	B	0.9–309	0.84	3.75	0.962	20.9
Icon, n = 43	A	1.4–130	0.59	4.38	0.987	5.4
	B	0.9–122	0.58	5.01	0.983	6.3
IMx, n = 43	A	1.4–130	0.71	1.59	0.984	6.0
	B	0.9–122	0.71	2.17	0.982	6.4

[a] Linear regression.

Discussion

The availability of specific monoclonal antibodies for the M and B subunits and MB isoenzyme of CK enabled the development of very specific and sensitive two-site immunoassays that measure CK-MB mass and use radiometric, fluorometric (*8*), chemiluminometric (*9*), or absorbance (*10*) detection systems. Correlation between measurements of CK-MB mass and activity is well established (*22*) and studies have successfully compared these two assay principles (*23–25*).

Time-resolved fluorescence has become an established analytical technique in the field of nonisotopic immunoassay and can achieve sensitivities equivalent to or better than those obtained with radiolabeled tracers (*14*). Here we describe a time-resolved immunofluorometric assay for CK-MB in serum. The assay is based on the formation of a complex involving CK-MB bound by a specific monoclonal antibody for CK-MB immobilized on the solid-phase and a biotinylated specific monoclonal antibody for the B subunit. The formed immunocomplex is quantified by a highly sensitive, recently described (*15*) streptavidin (SA)-based macromolecular complex of the approximate composition $SA(TG)_{3.3}BCPDA_{480}$, where TG is bovine thyroglobulin. This solid-phase complex is fluorescent and is measured on the surface of a microtiter well by a specially designed laser-excited, time-resolved fluorometer.

The proposed assay for CK-MB is highly sensitive, with a detection limit ≤0.4 µg/L, equivalent to that reported for Stratus (0.4 µg/L) but better than the reported detection limit of the MagicLite (0.8 µg/L) and the Icon (0.6 µg/L) assays (*19*). The reported detection limit of the IMx assay (0.2 µg/L) is superior (*21*). Only 25 µL of sample is required for a single assay. The assay is free from positive and negative interferences by CK-MM (up to 5000 µg/L) and CK-BB (up to 1000 µg/L), and calibration curves are linear to CK-MB concentrations of 100 µg/L. Serum dilution curves were linear over a wide range.

We have developed two different protocols, with total incubation times of either 30 or 60 min. Comparison studies with other commercially available immunoassays reveal good correlation although the absolute values differ, especially with the IMx and Icon assays. The reason for the discrepancies is unknown. We conclude that standardization must play a role because our assay is in close agreement with the Stratus assay, the method we used to assign values to our standards.

Our assay design, which incorporates a specific anti-CK-MB coating antibody and a specific anti-CK-B subunit antibody, is theoretically the most preferable. Assays that use anti-CK-B subunit coating antibody (the Icon assay) may suffer negative interference if high concentrations of CK-BB are present in serum. Also, use of anti-CK-B subunit detection antibody is preferable to the use of anti-CK-M subunit detection antibody (as used in the IMx assay) because of better specificity. The presence of high amounts of CK-MM in serum may cause problems if the coating antibody is not highly specific for CK-MB or if there is nonspecific binding of CK-MM.

References

1. Lang H, Würzburg U. Creatine kinase, an enzyme of many forms [Review]. Clin Chem 1982;28:1439–47.
2. Moss DW, Henderson AR, Kachmar JF. Enzymes. In: Tietz NW, ed. Textbook of clinical chemistry. Philadelphia: Saunders, 1986:619–763.
3. Lott JA, Stang JM. Serum enzymes and isoenzymes in the diagnosis and differential diagnosis of myocardial ischemia and necrosis [Review]. Clin Chem 1980;26:1241–50.
4. Kallner A, Sylven C, Brodin U, Loogna E, Svenhamn K. Early diagnosis of acute myocardial infarction. A comparison between chemical predictors. Scand J Clin Lab Invest 1989;49:633–9.
5. Koch TR, Mehta UJ, Nipper HC. Clinical and analytical evaluation of kits for measurements of creatine kinase isoenzyme MB. Clin Chem 1986;32:186–91.
6. Stein W, Bohner J, Steinhart R, Eggstein M. Macro creatine kinase: determination and differentiation of two types by their activation energies. Clin Chem 1982;28:19–24.
7. Wu AH, Bowers GN. Evaluation and comparison of immunoinhibition and immunoprecipitation methods for differentiating MB from BB and macroforms of creatine kinase isoenzymes in patients and healthy individuals. Clin Chem 1982;28:2017–21.
8. Goodnow T, LeBlanc M, Koshi K, et al. Radial partition immunoassay: quantitating CK-MB levels in human serum [Abstract]. Clin Chem 1987;33:988.
9. Piran U, Kohn DW, Uretsky LS, et al. Immunochemiluminometric assay of creatine kinase MB with a monoclonal antibody to the MB isoenzyme. Clin Chem 1987;33:1517–20.
10. Wu AHB, Gornet TG. Evaluation of an immunoenzymometric assay for CK-MB using the Icon format [Abstract]. Clin Chem 1989;35:1121.
11. Shah VD. Immunochemical test for CK-MB isoforms [Letter]. Clin Chem 1989;35:2157–8.
12. Thompson RJ, Jackson AP, Langlois N. Circulating antibodies to mouse monoclonal immunoglobulins on normal subjects—incidence, species specificity, and effects in a two-site assay for creatine kinase-MB isoenzyme. Clin Chem 1986;32:476–81.
13. Butch AW, Goodnow TT, Brown WS, McClellan A, Kessler G, Scott MG. Stratus automated creatine kinase–MB assay evaluated: identification and elimination of falsely increased results associated with a high molecular mass form of alkaline phosphatase. Clin Chem 1989;35:2048–53.
14. Diamandis EP. Immunoassays with time-resolved fluorescence spectroscopy: principles and applications [Review]. Clin Biochem 1988;21:139–50.
15. Morton RC, Diamandis EP. A streptavidin-based macromolecular complex labeled with an europium chelator suitable for time-resolved fluoroimmunoassay applications. Anal Chem 1990; in press.
16. Evangelista RA, Pollak A, Allore B, Templeton EF, Morton

RE, Diamandis EP. A new europium chelate for protein labeling and time-resolved fluorometric applications. Clin Biochem 1988;21:73–8.
17. Chan MA, Bellem AC, Diamandis EP. Time-resolved immunofluorometric assay for alpha-fetoprotein in serum and amniotic fluid with a novel detection system. Clin Chem 1987;33:2000–3.
18. Diamandis EP, Morton RC, Reichstein E, Khosravi MJ. Multiple fluorescence labeling with europium chelators. Application to time-resolved fluoroimmunoassays. Anal Chem 1988;61:48–53.
19. Wu AHB, Gornet TG, Harker CC, Chen HL. Role of rapid immunoassays for urgent ("stat") determinations of creatine kinase isoenzyme MB. Clin Chem 1989;35:1752–6.
20. Chapelle JP, Allaf ME. Automated quantification of creatine kinase MB isoenzyme in serum by radial partition immunoassay, with use of the Stratus analyzer. Clin Chem 1990;36:99–101.
21. Brandt DR, Gates RC, Eng KK, et al. Quantifying the MB isoenzyme of creatine kinase with the Abbott "IMx" immunoassay analyzer. Clin Chem 1990;36:375–8.
22. Eisenberg PR, Shaw D, Schaab C, Jaffe AS. Concordance of creatine kinase-MB activity and mass. Clin Chem 1989;35:440–3.
23. Chan DW, Taylor E, Frye R, Blitzer R. Immunoenzymometric assay for creatine kinase MB with subunit-specific monoclonal antibodies compared with an immunochemical method and electrophoresis. Clin Chem 1985;31:465–9.
24. Apple F, Preese L, Bennett R, Fredrickson A. Clinical and analytical evaluation of two immunoassays for direct measurement of creatine kinase MB with monoclonal anti-CK-MB antibodies. Clin Chem 1988;34:2364–7.
25. Delanghe JR, De Mol AM, Buyzere ML, De Scheerder IK, Wieme RJ. Mass concentration and activity concentration of creatine kinase isoenzyme MB compared in serum after acute myocardial infarction. Clin Chem 1990;36:149–53.