Clinical Chemistry 46:10
1604–1609 (2000)

Enzymes and Protein Markers

Clinical Evaluation of the First Medical Whole Blood, Point-of-Care Testing Device for Detection of Myocardial Infarction

Fred S. Apple,[1*] F. Philip Anderson,[2] Paul Collinson,[3†] Robert L. Jesse,[2] Michael C. Kontos,[2] M. Andrew Levitt,[4] Elizabeth A. Miller,[1] and MaryAnn M. Murakami[1]

Background: **Validation of whole blood, point-of-care testing devices for monitoring cardiac markers to aid clinicians in ruling in and ruling out myocardial infarction (MI) is necessary for both laboratory and clinical acceptance.**

Methods: **This study evaluated the clinical diagnostic sensitivity and specificity of the First Medical Cardiac Test device operated by nursing and laboratory personnel that simultaneously measures cardiac troponin I (cTnI), creatine kinase (CK) MB, myoglobin, and total CK on the Alpha Dx analyzer in whole blood for detection of MI. Over a 6-month period, 369 patients initially presenting to the emergency department with chest pain were evaluated for MI using modified WHO criteria. Eighty-nine patients (24%) were diagnosed with MI.**

Results: **In whole blood samples collected at admission and at 3- to 6-h intervals over 24 h, ROC curve-determined MI decision limits were as follows: cTnI, 0.4 μg/L; CKMB, 7.0 μg/L; myoglobin, 180 μg/L; total CK, 190 μg/L. Based on peak concentrations within 24 h after presentation, the following sensitivities (± 95% confidence intervals) were found: cTnI, 93% ± 5.5%; myoglobin, 81% ± 9.7%; CKMB, 90% ± 6.3%; total CK, 86% ± 7.5%. Sensitivities were maximal at >90% for both cTnI and CKMB at >12 h in MI patients, without differences between ST-segment elevation and non-ST-segment elevation MI patients.**

Conclusions: **The First Medical point-of-care device provides cardiac marker assays that can be used by laboratories and clinicians in a variety of hospital settings for ruling in and ruling out MI.**

The evaluation of patients with acute chest pain in emergency departments or during hospitalization often is a diagnostic challenge to physicians. Biochemical markers of myocardial injury are useful in aiding clinicians in confirming and ruling out the diagnosis of myocardial infarction (MI)[5] in both patient groups with and without a diagnostic electrocardiogram *(1, 2)*. In patients with unstable angina or acute coronary syndromes, where the electrocardiogram often fails to provide conclusive diagnostic information, biochemical markers, specifically cardiac troponin I (cTnI) and T have become reliable risk predictors of adverse short- and long-term outcomes *(3–5)*. Technological improvements in instrumentation have allowed the development of rapid, whole blood analysis of single or simultaneous multiple markers of myocardial injury in both qualitative *(3, 6)* and quantitative *(7–9)* formats. These systems have been designed to provide testing capabilities in the central laboratory, in satellite laboratories, or close to the bedside, by either laboratory or clinical personnel and have been designated as point-of-care (POC) testing.

Several acute coronary syndrome patient groups with varying clinical needs are targets for implementation of

[1] Hennepin County Medical Center, Department of Laboratory Medicine and Pathology, Minneapolis, MN 55415.
[2] Medical College of Virginia, Departments of Pathology and Medicine, Richmond, VA 23225.
[3] Mayday University Hospital, Surrey CR7 7YE, United Kingdom.
[4] Alameda County Medical Center–Highland Campus, Department of Emergency Medicine, Oakland, CA 94602.
*Address correspondence to this author at: Hennepin County Medical Center, Clinical Laboratories 812, 701 Park Ave., Minneapolis, MN 55415. Fax 612-904-4229; e-mail fred.apple@co.hennepin.mn.us.
†Current affiliation: St. George's Hospital and Medical School, London SW17 0QT, United Kingdom.
Received December 15, 1999; accepted July 17, 2000.

[5] Nonstandard abbreviations: MI, myocardial infarction; cTnI, cardiac troponin I; POC, point of care; FM, First Medical; CKMB, creatine kinase MB; and CI, confidence interval.

POC testing of markers of myocardial injury, including patients with acute MI (ST-segment elevation and depression, Q-wave and non-Q-wave or non-ST-segment elevation) and patients with noncardiac chest pain. At least one report has documented that a fast-track POC marker testing protocol was successful for a rapid rule-out protocol with cost reductions *(10)*. The purpose of this study was to clinically evaluate the diagnostic sensitivity and specificity of the First Medical (FM) Cardiac Panel test device *(11)* for the quantitative, simultaneous measurement of myoglobin, creatine kinase MB (CKMB) mass, cTnI, and total CK mass in whole blood in patients presenting with ischemic chest pain to rule in or rule out MI.

Materials and Methods

This study was performed at four sites: by nursing staff in the coronary care unit at Hennepin County Medical Center, Minneapolis, MN; by nursing staff in the emergency department at Alameda County Medical Center–Highland Campus, Oakland, CA; by laboratory technology staff in the central laboratory at the Medical College of Virginia, Richmond, VA; and by nursing staff in the coronary care unit at Mayday University Hospital, Thornton Heath, Surrey, United Kingdom. Staff at all sites were initially trained by representatives of FM regarding instrument operation and maintenance before the start of the study. All sites obtained approval for human subject research from the respective institutional review boards.

PATIENT SAMPLES

Specimens from 166 apparently healthy individuals (68 males and 98 females) were used to estimate the reference interval for all four markers evaluated on the FM Alpha Dx system, including myoglobin, CKMB mass, cTnI, and total CK mass. None of the healthy subjects had evidence of cardiac, renal, or skeletal muscle disease. The median ages for males and females were 28 years (range, 20–51 years) and 37 years (range, 20–62 years), respectively. Serial whole blood (EDTA) specimens (n = 1550) from 369 acute coronary syndrome patients (211 males and 158 females) presenting with chest pain suggestive of myocardial ischemia were also included, of which 89 had acute MI. The median ages for males and females were 58 years (range, 25–93 years) and 60 years (range, 30–92 years), respectively. The diagnosis of acute MI was made according to modified WHO criteria in which two of three of the following were present: chest pain duration >20 min, ST-segment elevation on the electrocardiogram, or increased cardiac markers *(12–14)*. The WHO criteria were considered modified because at least one site used cTnI as their routine biomarker *(13, 14)*. An increased cardiac marker was defined as any one of the serial specimens with an increased concentration above the diagnostic decision cutoff noted below.

A minimum of three serial specimens were collected, at admission and at approximately 3- to 6-h intervals to 24 h, depending on each hospital's protocol. Whole blood specimens were analyzed within 30 min of collection on the Alpha Dx system. Paired serum specimens (frozen at below −20 °C) were analyzed for each cardiac marker for comparison in the central laboratory at each of the four participating institutions on the following analyzers (diagnostic decision cutoffs): Dade Stratus II myoglobin (110 μg/L); Dade Stratus II CKMB (7.0 μg/L); Dade Stratus II cTnI (1.5 μg/L) or Beckman Access cTnI (0.15 μg/L); Ortho Clinical Diagnostics Vitros total CK (men, 300 U/L; women, 200 U/L).

EVALUATION OF CUTOFF CONCENTRATIONS

The 97.5th percentiles for the apparently healthy individuals and the non-MI patients were calculated. To determine the diagnostic cutoffs for acute MI patients, we performed ROC curve analysis for the Alpha Dx assays. The relationship between each concentration measured by the Alpha Dx vs the comparative device was determined using specimens whose Alpha Dx concentrations fell within the dynamic range of each assay.

ANALYTICAL PERFORMANCE

The minimum detectable concentration for each FM assay was defined as the analyte concentration corresponding to the mean + 2 SD from the response of 20 replicates of the zero calibrator. Imprecision was studied across sites, using frozen serum pools at two concentrations, over 20 days and with three lots of FM test disks and a single calibration per Alpha Dx instrument.

Alpha Dx SYSTEM

The Alpha Dx System, a fluorescence immunoassay platform that integrates automated solid-phase sandwich immunoassay capabilities with fluorescence detection, provides quantitative measurement of four cardiac markers: myoglobin, total CK mass, CKMB mass, and cTnI *(11)*. The system consists of test disks, safe-T-coupler units, a fluid cassette, and a calibration diskette. The test disk contains all required test-specific reagents, along with bilevel quality controls for each test in stabilized dry form. The safe-T-coupler unit is used to load a primary blood collection tube into the analyzer, where 80 μL of sample is automatically metered from the sample tube into the sample chamber, through the safe-T-coupler unit,

Table 1. Total imprecision of the FM Alpha Dx cardiac panel test device over 20 days using frozen serum samples.[a]

	Control 1		Control 2	
	Mean, μg/L	CV, %	Mean, μg/L	CV, %
Myoglobin	106	7.7	400	6.2
Total CK mass	193	8.4	745	4.0
CKMB mass	8.4	6.3	133	5.2
cTnI	0.30	7.4	2.38	8.8

[a] n = 146–152 for each control tested.

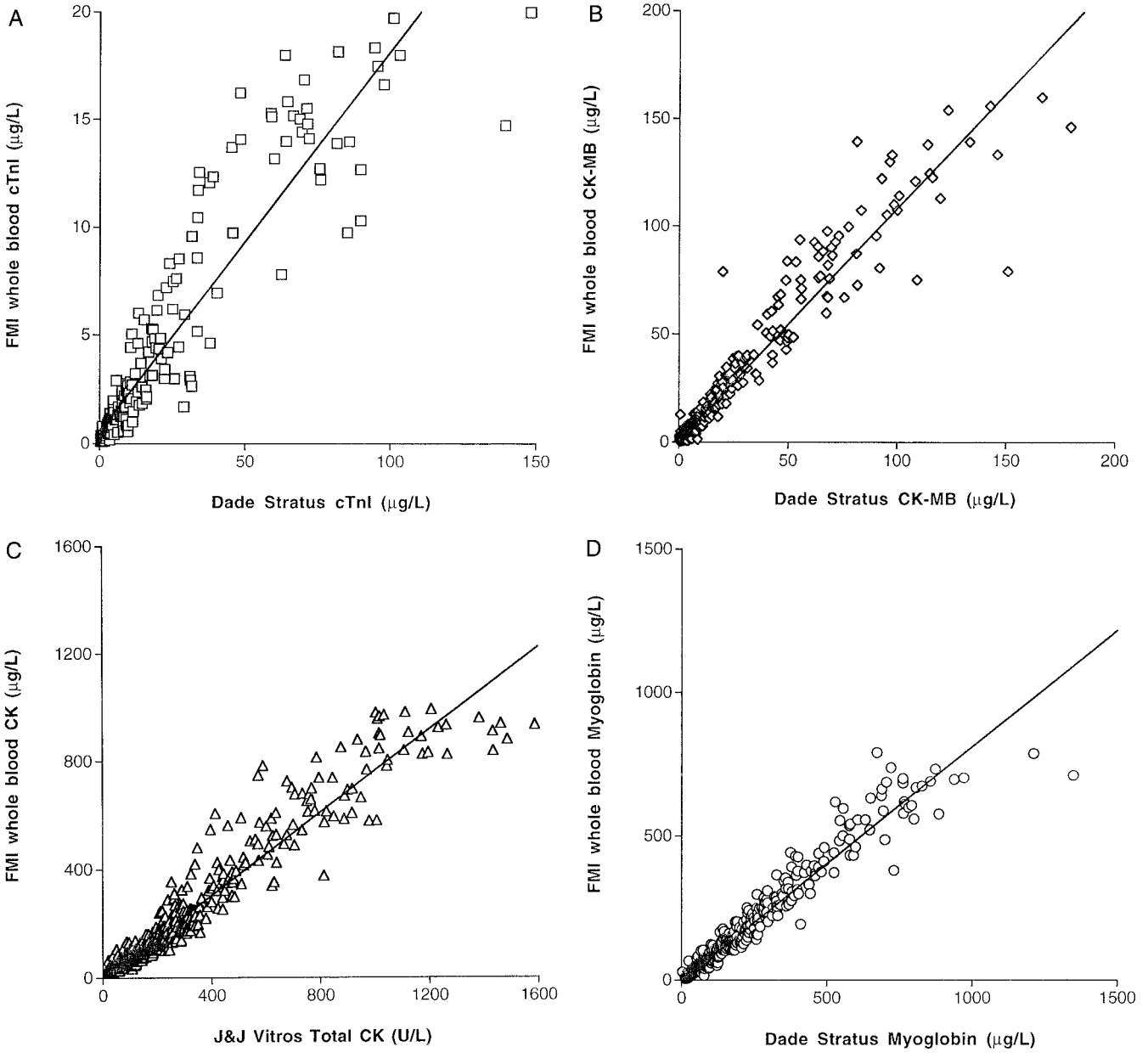

Fig. 1. Correlation of FM Alpha Dx test panel over the dynamic range of all four assays.

(*A*), cTnI vs Stratus: $y = 0.18x + 0.56$; $r = 0.92$; n = 226; (*B*), CKMB vs Stratus: $y = 1.07x + 1.1$; $r = 0.97$; n = 676; (*C*), total CK vs Vitros: $y = 0.77x - 1.0$; $r = 0.97$; n = 923; (*D*), myoglobin vs Stratus: $y = 0.81x - 2.0$; $r = 0.98$; n = 995. The *solid line* is the regression line. All units are in μg/L except the Vitros total CK, which is in U/L.

where it is mixed with the fluorescein-labeled antibodies. A minimum of 1.0 mL of blood is needed for analysis. The fluid cassette contains a stabilized, buffered detergent solution that is used for both the rehydration of dried reagents and protocol washes. The calibration diskette contains lot-specific calibration information, limits for system quality control, and expiration dating for each lot of test disks. The system is designed for STAT operation, with assay results available in <20 min. Biohazard waste and assay fluids are safely contained within the test disc and safe-T-coupler unit. The test disc built-in bilevel controls are processed in parallel with the patient sample. External quality-control procedures are not required.

In a typical sandwich fluorescent immunoassay in the test device, antigens are detected by binding to two distinct antibodies, one immobilized on a solid phase and the other coupled to a fluorescent label. The test disc uses three antibodies: a solid-phase antibody, a hapten (fluorescein)-labeled antibody, and a fluorescently labeled anti-hapten (fluorescein) antibody. The three-antibody system provides enhanced signal amplification and improved assay sensitivity. The test disc consists of three

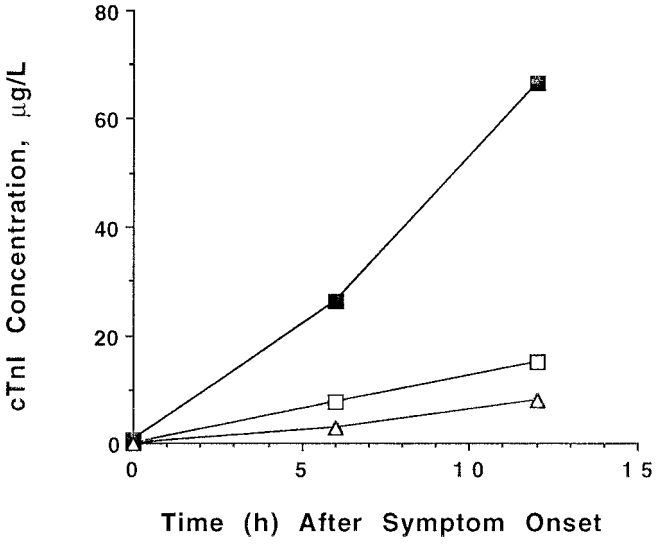

Fig. 2. FM Alpha Dx cTnI (□) vs Stratus cTnI (■) and Access cTnI (△) measured in serial specimens from a representative acute MI patient collected from admission.

zones, one for the test sample and two for the quality controls. In addition, each disc contains a hematocrit chamber and a waste ring. All zones have four spatially distinct but fluid-connected features that function as analyte capture sites within a shared reaction chamber. In all four assays, fluorescence intensity is proportional to the amount of bound label and, therefore, to the concentration of analyte in the sample, measured after excitation by a 640 nm diode laser in the analyzer fluorometer. The packed cell volume, obtained form the hematocrit procedure, is used to convert the measured whole blood sample result to a matched serum sample. All reactions are temperature controlled at 37 °C.

STATISTICAL COMPARISON

To compare the Alpha Dx System with each laboratory's established devices, Passing-Bablock linear regression analysis was performed on single tests from each sample over the dynamic range of both assays. Values below the minimum detectable concentration of either assay were excluded from the data analysis. ROC curves were analyzed for each assay *(15)*. Diagnostic sensitivity and specificity data are presented with 95% confidence intervals (95% CIs) and compared between assays by the McNemar test. All statistical tests were two-tailed, with significance set at $P < 0.05$. SAS Ver. 6.09 software was used for all statistical analyses. The data presented here are the basis of materials submitted to the Food and Drug Administration for 510K approval of the four cardiac markers on the FM Alpha Dx instrument.

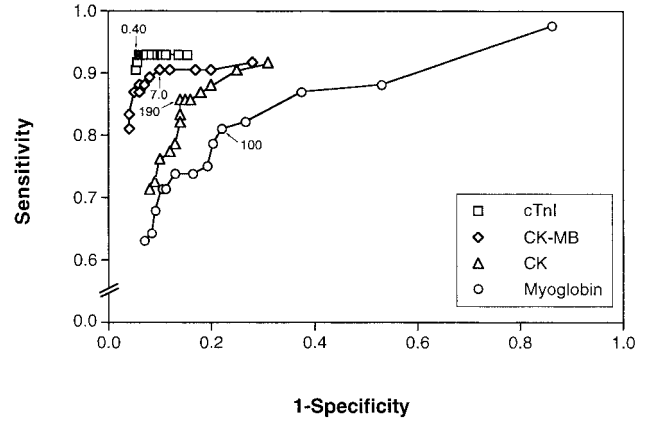

Fig. 3. ROC curves for the Alpha Dx cTnI, CKMB, myoglobin, and total CK assays.

The *arrows* indicate the medical decision cutoff points for MI diagnosis for each assay.

Results

ANALYTICAL PERFORMANCE

As shown in Table 1, the total imprecision (CV) of the Alpha Dx assays was 4.0–8.8% for the two frozen serum controls. The lower limits of detection were as follows: myoglobin, 5 μg/L; CK, 10 μg/L; CKMB, 0.5 μg/L; cTnI, 0.09 μg/L.

Regression analysis statistics are demonstrated in Fig. 1. Samples were from paired cardiac marker measurements between the Alpha Dx (whole blood) and the comparative devices (serum) in subsets from the total specimens (n = 1550) collected from 276 suspected acute MI patients and 134 healthy individuals over the dynamic range of each assay. Comparison of the Alpha Dx cTnI with the Stratus cTnI assay ($y = 0.18x + 0.56$; $r = 0.92$; n = 226) and Access cTnI assay ($y = 1.44x + 0.96$; $r = 0.88$; n = 244) showed substantially different slopes: 0.18 and 1.44, respectively. Bias plots revealed a proportional (38%) increasing positive bias across the range of concentrations tested for by the Stratus II cTnI compared with the Alpha Dx cTnI. Bias plots for total CK, myoglobin, and CKMB demonstrated random scatter at the upper end of linearity for each marker between assays (data not shown). A representative time-vs-cTnI concentration profile for one MI patient, shown in Fig. 2, demonstrates the different absolute and relative concentrations that are observed among the different cTnI assays.

CLINICAL PERFORMANCE

Fig. 3 shows cTnI, CKMB, myoglobin, and total CK ROC curves determined using peak concentrations over the 24 h after presentation from the 369 ischemic chest pain patients. The sensitivities and specificities (95% CIs), respectively, at decision cutpoints were as follows: 0.4 μg/L cTnI, 93% (87.5–98.5%) and 94% (91.3–96.7%); 7.0 μg/L CKMB, 90% (82.7–96.3%) and 90% (86.6–93.4%); 100 μg/L myoglobin, 81% (71.5–90.5%) and 81% (77.3–84.7%); 190 μg/L total CK, 86% (78.5–93.5%) and 86% (81.9–90.1%). There was a 92% concordance between paired results for diagnosis between the FM cTnI and FM CKMB compared with the Dade cTnI and Dade CKMB assays, respectively. The area under the cTnI ROC curve (0.919) was significantly larger ($P < 0.05$) compared with CKMB

(0.890), myoglobin (0.541), and total CK (0.856). There were no statistical differences for the areas under the ROC curves for the FM cTnI assay compared with the Dade cTnI assay (0.925), whose cutpoint of 1.5 μg/L gave a 93% (95% CI, 87.4–98.6%) sensitivity and 93% (90.1–95.9%) specificity.

For sensitivity and specificity calculations, McNemar χ^2 analysis showed no statistical differences in concordances between the FM CKMB and Stratus CKMB assays, the FM cTnI and Stratus cTnI assays, or the FM myoglobin and Stratus myoglobin assays (data not shown). Based on peak values over the 24 h after onset of symptoms, the sensitivities (± SD) of all four markers on the FM device were not statistically different in MI patients with ST-segment elevation (n = 60) and non-ST-segment elevation (n = 29) infarctions, respectively: myoglobin, 76% ± 10% and 58% ± 18%; CK, 87% ± 8% and 80% ± 15%; cTnI, 93% ± 5% and 90% ± 9%; CKMB, 90% ± 7% and 83% ± 14%. cTnI demonstrated >90% sensitivity compared with 77–90% sensitivity for myoglobin, total CK, and CKMB. Fig. 4 shows the clinical sensitivity of the FM cTnI assay for ST-segment elevation MI patients (n = 60) compared with non-ST-segment elevation MI patients (n = 29) based on time intervals from onset of symptoms. Myoglobin was the most sensitive in the early 0–4 h time frame (61%), compared with total CK (43%), CKMB (50%), and cTnI (29%). At 5–11 h, the sensitivity was 90% for CKMB and 75% for cTnI. At 12–23 h, the sensitivities for both CKMB and cTnI were >90%. Also using peak values over the 24-h period after onset of chest pain, specificities between ST-segment elevation and non-ST-segment elevation MIs were not statistically different from the overall specificities noted above.

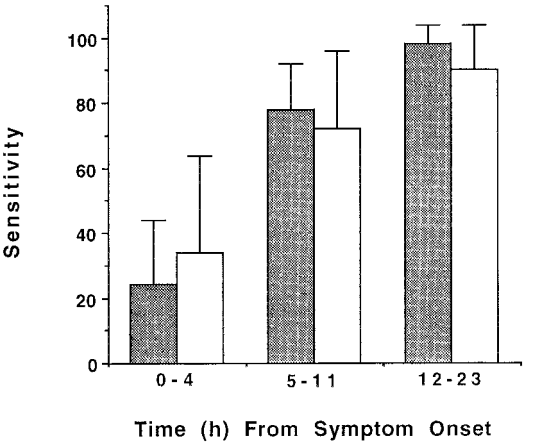

Fig. 4. Sensitivity (%) of FM Alpha Dx cTnI assay by hours after symptom onset for detecting ST-segment elevation (*filled columns*; n = 60) and non-ST-segment elevation (*open columns*; n = 29) infarctions.
Bars, 95% CIs.

Discussion

This study demonstrates that a quantitative, whole blood POC testing device, operated by either laboratory or non-laboratory (nursing) personnel, was comparable to established laboratory devices measuring cardiac markers for detection of MI. The data show that simultaneous quantification of whole blood myoglobin, total CK mass, CKMB mass, and cTnI in patients presenting with chest pain had acceptable concordances with central laboratory instrumentation that measured markers individually in serum. Sensitivity calculations for the FM Alpha Dx panel demonstrated the expected temporal increases over the 24-h period after admission with >90% sensitivity for cTnI at peak concentrations. Furthermore, there were no diagnostic sensitivity differences when patients were divided according to ST-segment elevation and non-ST-segment elevation MIs (Fig. 4), with maximum sensitivity 12–23 h after presentation at >90% in both MI groups when cTnI was used. Although not statistically significant, sensitivities for CKMB in both groups were lower. It should be noted that MI patients who may have received thrombolytic therapy from the ST-segment elevation MI group were not separated for analysis.

cTnI, a cardiac-specific protein that is challenging CKMB as a new marker for MI detection *(1, 13, 14, 16)*, demonstrated concentration differences when the FM cTnI assay was compared with the Dade Stratus II and Beckman Access assays, as shown in Figs. 1 and 2. The two- to sevenfold concentration differences in assays substantiate the lack of appropriate standardization between assay systems *(16–18)*. This underlines the need for individual laboratories and hospitals to establish their own decision cutpoints for each cTnI assay used for medical decisions if appropriate peer-reviewed studies are not available in the literature.

In addition to CKMB mass and cTnI, myoglobin and total CK mass concentrations are also obtained on the Alpha Dx panel. Although not cardiac specific, myoglobin was shown to be the most sensitive marker early after presentation in chest pain, confirming what is established in the literature *(19)*. This study did not address the role of myoglobin as a predictor for ruling out MI *(20)*. Unique to this assay system is the measurement of total CK mass. Similar to the myoglobin, CKMB, and cTnI assays, the total CK mass assay demonstrated acceptable concordance with the total CK activity assays (Fig. 1). The range in values between assays found in the regression analysis was likely because the comparison assay measures activity (Vitros), whereas the Alpha Dx is a mass immunoassay. However, it is not clear what clinical utility the addition of this marker will add, if any, to the rule-in/rule-out process for MI. Furthermore, the range in CKMB values found in the regression analysis (Fig. 1) was likely attributable to the lack of standardization between the two mass assays. All four assays on the Alpha Dx panel were found to be analytically acceptable for use in clinical laboratory testing.

Although cardiac markers are often not necessary for the diagnosis of acute MI in patients presenting with an

ST-segment elevation MI, markers are essential criteria for the diagnosis of non-ST-segment elevation MI *(21)*. The findings in our study showed no statistical differences in sensitivity for any of the four markers between patients with ST-segment elevation and non-ST-segment elevation infarctions based on peak concentrations within 24 h after presentation or in serial determinations for 0–4, 5–11, or 12–23 h groupings after presentation. However, there was a trend toward a lower clinical sensitivity in the non-ST-segment elevation infarction patients, as shown in Fig. 4. This may be influenced by the central laboratory assay cutpoints used for each assay. We purposely separated out the clinical sensitivities for the cardiac markers by ST-segment elevation and non-ST-segment elevation MI to emphasize the point that markers such as cTnI and CKMB, which are heavily relied on in the diagnostic criteria in the non-ST-segment elevation MI diagnosis, are diagnostically similar to ST-segment elevation MI in clinical utilization. Finally, no long- or short-term prognostic information was obtained in this study for either the MI patients or the patients with increased cTnI concentrations (minor myocardial damage) who ruled out for MI because it was not part of our study design.

In conclusion, the FM Alpha DX Cardiac Panel provides a platform for the simultaneous, quantitative measurement of total CK mass, CKMB mass, myoglobin, and cTnI in either whole blood or serum in <20 min. This is relevant in medical centers that are unable to support a rapid (<1 h) turnaround time from the central laboratory *(1)*. The diagnostic sensitivity and specificity of each test panel are equivalent to other commercially available assay systems. The FM system provides a POC testing system suitable for use by laboratory or non-laboratory personnel in a coronary care unit, emergency department, or central laboratory setting in both rural hospitals and major medical centers.

This study was partially funded by First Medical, Inc., Mountain View, CA.

References

1. Wu AHB, Apple FS, Gibler WB, Jesse RL, Warshaw MM, Valdes R Jr. National Academy of Clinical Biochemistry standards of laboratory practice: recommendations for use of cardiac markers in coronary artery diseases. Clin Chem 1999;45:1104–21.
2. Wu AHB, ed. Cardiac markers. Totowa, NJ: Humana Press, 1998: 300 pp.
3. Hamm CW, Goldmann BU, Heeschen C, Kreymann G, Berger J, Meinertz T. Emergency room triage of patients with acute chest pain by means of rapid testing for cardiac troponin T or troponin I. N Engl J Med 1997;337:1648–53.
4. Ohman EM, Armstrong PW, Christenson RH, Granger CB, Katus HA, Hamm CW. Cardiac troponin T levels for risk stratification with admission cardiac troponin T levels in acute myocardial ischemia. The GUSTO IIa Investigators. N Engl J Med 1996;335:1333–41.
5. Antman EM, Tanasijevic MJ, Thompson B, Schactman M, McCabe CH, Cannon CP. Cardiac-specific troponin I levels to predict the risk of mortality in patients with acute coronary syndromes. N Engl J Med 1996;335:1342–9.
6. Antman EM, Grudzien C, Mitchell RN, Sacks DB. Detection of unsuspected myocardial necrosis by rapid bedside assay for cardiac troponin T. Am Heart J 1997;133:596–8.
7. Apple FS, Christenson RH, Valdes R Jr, Andriak AJ, Berg A, Duh SH, et al. Simultaneous rapid measurement of whole blood myoglobin, creatine kinase MB, and cardiac troponin I by the Triage cardiac panel for detection of myocardial infarction. Clin Chem 1999;45:199–205.
8. Muller-Bardorff M, Rauscher T, Kampmann M, Schoolman S, Mangold D, Remppis A, Katus HA. Quantitative bedside assay for cardiac troponin T: a complementary method to centralized laboratory testing. Clin Chem 1999;45:1002–8.
9. Heeschen C, Goldmann BU, Langenbrink L, Matschuck G, Hamm CW. Evaluation of a rapid whole blood ELISA for quantification of troponin I in patients with acute chest pain. Clin Chem 1999;45: 1789–96.
10. Collinson PO. Economic aspects of new biochemical markers for the detection of myocardial damage. In: Kaski JC, Holt SW, eds. Myocardial damage: early detection by novel biochemical markers. London: Kluwer Academic Publishers, 1998:173–87.
11. Miller EA, Apple FS, Collinson R, Anderson FP, Jesse RL, Kontos MC, Levitt MA. Clinical evaluation of the Alpha Dx cardiac panel for total CK mass, CKMB mass, cardiac troponin I, and myoglobin for detection of myocardial infarction [Abstract]. Clin Chem 1998;44: A118.
12. Tunstall-Pedoe H, Kuulasmaa K, Amougel P. Myocardial infarction and coronary deaths on the World Health Organization MONICA project. Circulation 1994;90:583–612.
13. Falahati A, Sharkey SW, Christensen D, McCoy M, Miller E, Murakami MA, Apple FS. Implementation of serum cardiac troponin I as marker for detection of acute myocardial infarction. Am Heart J 1999;137:332–7.
14. Apple FS, Falahati A, Paulsen PR, Miller EA, Sharkey SW. Improved detection of minor ischemic myocardial injury with measurement of serum cardiac troponin I. Clin Chem 1997;43:2047–51.
15. Henderson AR, Bhayana V. A modest proposal for the consistent presentation of ROC plots in Clinical Chemistry. Clin Chem 1995;41:1205–6.
16. Apple FS. Tissue specificity of cardiac troponin I, cardiac troponin T and creatine kinase MB. Clin Chim Acta 1999;284:151–9.
17. Apple FS. Clinical and analytical standardization issues confronting cardiac troponin I. Clin Chem 1999;45:18–20.
18. Wu AHB, Feng YJ, Moore R, Apple FS, McPherson PH, Buechler KF, Bodor G. Characterization of cardiac troponin subunit release into serum following acute myocardial infarction, and comparison of assays for troponin T and I. Clin Chem 1998;44:1198–208.
19. Zimmerman J, Fromm R, Meyer D, Boudreaux A, Wun CCC, Smalling R. Diagnostic marker cooperative study for the diagnosis of myocardial infarction. Circulation 1999;99:1671–7.
20. Montague C, Kircher T. Myoglobin in the early evaluation of acute chest pain. Am J Clin Pathol 1995;104:472–6.
21. Stubbs P, Collinson P, Moseley D, Greenwood T, Noble M. Prognostic significance of admission troponin T concentrations in patients with myocardial infarction. Circulation 1996;94:1291–7.