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

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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.

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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) 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.
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Received December 15, 1999; accepted July 17, 2000.

1 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>
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<th>Table 1. Total imprecision of the FM Alpha Dx cardiac panel test device over 20 days using frozen serum samples.*</th>
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<td><strong>Control</strong></td>
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<tr>
<td>Mean, µg/L</td>
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<td>Myoglobin</td>
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<td>Total CK mass</td>
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<td>CKMB mass</td>
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* n = 146–152 for each control tested.
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

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**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.
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 from 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.

**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.
ties noted above.

ST-segment elevation and non-ST-segment elevation MIs
24-h period after onset of chest pain, specificities between
and cTnI were
75% for cTnI. At 12–23 h, the sensitivities for both CKMB
(29%). At 5–11 h, the sensitivity was 90% for CKMB and
compared with total CK (43%), CKMB (50%), and cTnI
the most sensitive in the early 0 – 4 h time frame (61%),
time intervals from onset of symptoms. Myoglobin was
non-ST-segment elevation MI patients (n = 60) compared with
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 specifici-

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 be-
tween assay systems (16–18). This underlines the need for
individual laboratories and hospitals to establish their
own decision cutoffs 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 concor-
dance 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 activ-
ity (Vitros), whereas the Alpha Dx is a mass immunoas-
say. 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