Evaluation of Stratus® CS Stat Fluorimetric Analyser for Measurement of Cardiac Markers Troponin I (cTnI), Creatine Kinase MB (CK-MB), and Myoglobin

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Myoglobin, CK-MB, and Troponin I (cTnI) are cardiac muscle necrosis markers that are useful for detecting acute myocardial infarction (AMI). The Stratus® CS (Dade Behring, Inc.) is a discrete fluorimetric immunoassay analyser designed for the determination of the three cardiac markers from a single sample of whole blood or plasma. Overall analytical performances of the Stratus® CS provided by Dade Behring were evaluated according to the French Society of Clinical Biology guidelines. Within-run imprecision ($n = 20$) for the three parameters at three levels gave values under 5%, whereas CVs for between-run im-

precision ($n = 20$) were under 6%. The sensitivities were 0.03 $\mu\text{g/L}$ for cTnI and 0.4 $\mu\text{g/L}$ for CK-MB. Linearities extended from 0–50 $\mu\text{g/L}$ for cTnI, 0–140 $\mu\text{g/L}$ for CK-MB, and 1–900 $\mu\text{g/L}$ for myoglobin. The results, particularly those obtained on whole-blood samples, correlated well with those obtained on Stratus® II. We did not find any interference with haemolysis, icterus, or lipemia. The system was very easy to use, and fulfills the requirements for the analysis of the three cardiac markers in patients with acute chest pain in emergency situations. *J. Clin. Lab. Anal.* 15:314–318, 2001. © 2001 Wiley-Liss, Inc.

Key words: Troponin I; CK-MB; myoglobin; immunoassay

INTRODUCTION

The measurement of cardiac markers is becoming increasingly important in the evaluation of patients with acute chest pain by improving confidence in cardiac risk assessment. Myoglobin has proved to be an excellent early marker for the accurate diagnosis of acute myocardial infarction (AMI), but definitive markers such as creatine kinase MB (CK-MB) mass and cardiac Troponin I (cTnI) appear to be much more useful. In particular, cTnI has been shown to be more powerful in identifying patients with increased risk of cardiac events (1–3).

Several techniques have been developed to measure these three markers, but all have a long turnaround time. In this study we evaluated the Stratus® CS, a discrete fluorimetric immunoassay analyser, for the three cardiac markers on both whole blood and plasma.

MATERIALS AND METHODS

Apparatus and Reagents

The Stratus® CS (Dade Behring, Inc., Deerfield, IL) is designed to analyse closed routine sample tubes containing anticoagulated whole blood (collected in heparin lithium). A centrifugation step (15,000 g for 2 min) is incorporated into the system for separation of plasma from blood cells. Alter-

natively, the system can analyse a preprocessed plasma specimen, which must be placed into a sample cup. All of the reagents are included in specific test packs. Up to four different test packs can be introduced for each sample; thus, the three cardiac markers can be processed in parallel.

The assays are two-site immunoassays in which monoclonal antibodies are covalently linked to Starburst® (Dendritech Inc., Midland, MI) dendrimers (solid phase) or alkaline phosphatase (label). The dendrimers are well-defined polymers composed of polyamidoamine groups distinguished by uniformity in molecular size and shape. The dendrimer technology provides for better presentation and functionality of the capture antibody on the fiberglass solid phase surface (4). The analytical process consists of adding dendrimer coupled capture antibody onto the fiberglass solid support, and then adding centrifuged plasma. Thereafter, alkaline phosphatase-labeled antibody is added to the mixture, followed by the addition of substrate/wash reagent. Unbound labeled antibody is removed

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from the reaction zone, allowing the detection of fluorogenic product, which is directly related to the concentration of the cardiac marker protein in the sample. The first result is available in 3 min, and the next every 4 min.

Dilutions can be performed automatically by adding a test-specific dilution pack. The Stratus® CS (Dade Behring, Inc.) will automatically perform a calibration update using a Calpak® and three analyte-specific test packs. Information for calibration update is stored in the bar codes of the test packs. The Stratus® CS was evaluated relative to the Stratus® II, which uses the same process (identical specific monoclonal antibodies) except for the dendrimer technology.

Patient Samples

To evaluate the analytical performance of Stratus® CS, the plasma samples from 100 patients were analysed by both Stratus® CS and Stratus® II. The samples were dispensed in sample cups specific for each apparatus. cTnI, CK-MB mass, and myoglobin were quantified on both systems in parallel.

For the evaluation of the practicability of the Stratus® CS on primary tube samples, 50 whole-blood samples were collected on lithium heparin (vacutainer® tubes, Becton Dickinson, Franklin Lakes, NJ). A minimum volume of 3.0 ml of whole blood was required for processing.

All of the analyses were performed within 2 hr after collection.

Evaluation of Analytical Performance

The evaluation was made according to the Valtec protocol designed by the French Society of Clinical Biology (5,6). Statistics were recorded as recommended using PC Valtec 97 V.1.0.4 software.

Stratus® CS imprecision was examined by using three concentrations of quality control materials, including the three analytes (Liquicheck 1, 2, and 3 cardiac markers, Bio-Rad, Hercules, CA). They were analysed in duplicate with the same lot and unchanged calibration. To determine intraassay imprecision, each control was analysed

TABLE 1. Intrassay and interassay imprecision of the Stratus® CS for the three cardiac markers

Analyte	Intraassay (n = 20)			Interassay (n = 20)		
	Mean (µg/L)	SD	CV(%)	Mean (µg/L)	SD	CV (%)
cTnI	0.713	0.0159	2.2	0.705	0.0209	3.0
	4.087	0.0766	1.9	4.197	0.1067	2.5
	11.530	0.3026	2.6	11.385	0.1952	1.7
CK-MB	3.24	0.176	5.4	3.32	0.151	4.5
	13.64	0.472	3.5	13.80	0.408	3.0
	51.01	1.677	3.3	47.99	1.279	2.7
Myoglobin	62.5	2.65	4.2	58.9	3.73	6.3
	187.5	6.10	3.3	179.2	9.05	5.1
	439.1	11.32	2.6	394.1	19.57	5.0

CV, coefficient of variation; SD, standard deviation.

Fig. 1. Linearities of the assays for cTnI, CK-MB, and myoglobin on Stratus® CS.

Fig. 2. Correlation between Stratus® CS and Stratus® II for cTnI at low concentration (< 0.3 µg/L).

20 times in one series. To determine interassay imprecision (day to day), each control was tested on 20 consecutive days.

The detection limit was defined as the lowest concentration that can be differentiated from zero (mean $\pm$ 2 SD). For cTnI, this was evaluated by the analysis of 20 replicates of a pool obtained from five healthy patients at a concentration below the detection limit of the assay on Stratus® II: 0.35 µg/L.

For CK-MB, it was evaluated by the determination of the limit dilution of a pool at low CK-MB concentration as measured on Stratus® CS, giving values different from zero.

The limit of quantification was determined in serial dilutions of plasma with known cTnI, CK-MB, and myoglobin concentrations. For cTnI, the dilutions were undertaken with a cTnI negative plasma obtained from a healthy individual; for CK-MB and myoglobin, the dilutions were achieved in 0.15 mmol/L NaCl.

The correlation between the cTnI, CK-MB, and myoglobin measurements of the Stratus® CS and Stratus® II was compared in 100 plasma samples collected in Terumo® tubes.

Correlation between plasma samples (Terumo®) and whole blood samples (Becton Dickinson vacutainer®) was also compared in 50 samples; both types of tubes contained lithium heparin as anticoagulant.

The effect of haemolysis was tested by overloading a plasma pool with known cTnI, CK-MB, and myoglobin concentrations with 50 g/L haemoglobin (Sigma Chemical Co., St. Louis, MO). Five-step dilutional series were undertaken.

The influence of icterus was studied by enhancing bilirubin concentration up to 500 µmol/L (in terms of unconjugated bilirubin) using an icteric plasma specimen. For lipemia, we used an intralipid solution (Kabivitrum SA) enhancing triglycerides concentration up to 7.0 mmol/L.

RESULTS

The coefficients of variation for intraassay imprecision, as determined on three levels, ranged from 1.9–2.6% for cTnI, 3.3–5.4% for CK-MB mass, and 2.6–4.2% for myoglobin (Table 1). For interassay imprecision, the coefficients of variation were from 1.7–3.0% for cTnI, 2.7%–4.5% for CK-MB mass, and 5.0–6.3% for myoglobin.

We verified the linearities suggested by Dade Behring for the three analytes; they extended from 0–50 µg/L for cTnI, 0–140 µg/L for CK-MB, and 1–900 µg/L for myoglobin (Fig. 1).

Twenty measurements on the plasma at zero level (as defined in Materials and Methods) showed a mean cTnI concentration of 0.007 ± 0.005 µg/L, resulting in an analytical detection limit of 0.03 µg/L (representing the lowest concentration that can be distinguished from zero). Twenty measurements on the diluted plasma with low CK-MB concentration gave a mean CK-MB concentration of 0.37 ± 0.048 µg/L, resulting in an analytical detection limit of 0.4 µg/L. These results are identical with the limits given by the manufacturer. We did not verify the detection limit for myoglobin (1 µg/L, as stated by the manufacturer), because both the physiological and pathological values are significantly greater than this limit.

The Stratus® CS and Stratus® II results were compared by analysis of cTnI, CK-MB, and myoglobin; the correlation data are given in Table 2. There was no correlation observed when comparing cTnI results below 0.3 µg/L ($r^2 = 0.45$, Fig. 2). The data were also compared for whole blood and plasma samples; regression analysis did not indicate any difference between the results for the three analytes (Table 3).

Haemoglobin, bilirubin, and lipids did not influence the results for the three analytes. We did not find any interfer-

TABLE 2. Correlation between Stratus® CS and Stratus® II for the three cardiac markers

Analyte	n	Correlation coefficient	Deming regression	Comments
cTnI	100	0.997	$1.00x + 0.01$	Accepted ^a
CK-MB	100	0.992	$0.90x + 1.45$	Accepted ^a
Myoglobin	100	0.998	$1.04x + 2.58$	Accepted ^a

^aAccording to the recommendations of the French Society of Clinical Biology.

ence for cTnI, CK-MB, or myoglobin with haemoglobin up to 210 µmol/L, with bilirubin up to 500 µmol/L, and with triglycerides up to 7.0 mmol/L.

We then measured cTnI on hemolyzed plasma (n = 10) and compared the results with those obtained with Stratus® II (Table 4). The interference for cTnI found on Stratus® II (related to red blood corpuscles not eliminated by centrifugation) was not found on the Stratus® CS. The Stratus® CS displays second-generation assays with completely new formulations in comparison to Stratus® II, and its dendrimer technology would certainly explain the disappearance of haemolysis interference.

DISCUSSION

We evaluated the Stratus® CS for routine use in clinical chemistry or a chest pain unit over a 2-month period. The system presented excellent analytical characteristics whatever the analyte: low imprecision, a low detection limit (particularly for cTnI), and a good limit of quantification with acceptable imprecision.

Because identical antibodies and calibration fits are used in both Stratus® CS and Stratus® II, a high correlation was observed between both apparatus whatever the analyte. Nevertheless, no correlation was revealed for cTnI at low concentration (<0.3 µg/L), which can be explained by the low detection limit for the Stratus® CS (<0.03 µg/L) compared with 0.35 µg/L for Stratus® II. These striking differences might be related to the improved ELISA design of the Stratus® CS, which uses dendrimer technology. This low limit of detection may be particularly suitable in clinical situations such as risk stratification in cardiology and emergency units.

Moreover, quantitative results on whole-blood samples are available within 15 min after blood collection. The simplicity and convenience of the procedure, and the turnaround time

TABLE 3. Comparison between primary tubes (Becton Dickinson Vacutainer®) and plasma samples (Terumo® tubes) for the analysis of cardiac markers on Stratus® CS

Analyte	n	Correlation coefficient	Deming regression	Comments
cTnI	50	0.997	$0.96x + 0.07$	Accepted ^a
CK-MB	50	0.998	$0.97x + 0.06$	Accepted ^a
Myoglobin	50	0.997	$0.96x + 2.48$	Accepted ^a

^aAccording to the recommendations of the French Society of Clinical Biology.

TABLE 4. Influence of haemolysis on the determination of cTnI on Stratus® CS in comparison to Stratus® II

Degree of haemolysis	Stratus® II cTnI (µg/L)	Stratus® CS cTnI (µg/L)
+	0.4	0.03
+	0.5	0.03
+	0.5	0.03
+	0.9	0.01
++	0.9	0.03
++	0.7	0.05
++	1.1	0.02
+++	1.0	0.04
+++	1.2	0.03
+++	1.1	0.03

required to simultaneously quantify myoglobin, CK-MB, and cTnI, should recommend its use at the point of care by medical and paramedical staffs.

Finally, the Stratus® CS has proved an excellent immunoanalyser for the three cardiac markers cTnI, CK-MB, and myoglobin; in particular, it fulfills the requirements for the diagnosis of myocardial infarction and is well designed for the analysis of cardiac markers in patients with acute chest pain in emergency situations (7,8). Moreover, the increased sensitivity of cTnI Stratus® CS could help to detect more patients with unstable angina, microinfarction, or atypical injury, events with an increased risk of mortality (9,10).

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