Attachment 3

K961412

SUMMARY OF SAFETY AND EFFECTIVENESS

CK-MB METHOD FOR THE IMMUNO 1 SYSTEM

Listed below are a comparison of the performance between serum and plasma samples on the Immuno 1 CK-MB method (T01-3587-51). The information used in this summary of Safety and Effectiveness was extracted from the CK-MB method sheet (attachment) and from data on file at Bayer Corp.
The reagents, calibrators and software are the same regardless of whether serum or plasma is used as the sample.

INTENDED USE

This in vitro diagnostic procedure is a solid- phase enzyme immunoassay intended for the quantitative determination of CK-MB in human serum or plasma on the Technicon Immuno 1 system. When used in combination with other clinical data such as presenting symptoms and EKG values, measurement of CK-MB aids in the diagnosis of acute myocardial infarction.

ASSAY DESCRIPTION

The assay is an enzyme label sandwich assay using two monoclonal antibodies. A CK-MB specific antibody is labelled with fluorescein and the Fab' fragment of an antibody specific for the B subunit is labelled with alkaline phosphatase(ALP) The solid phase consists of a suspension of magnetizable particles coated with antibody to fluorescein (IMP reagent). Sample or calibrator, R1 reagent containing fluorescein - antibody conjugate, R2 reagent containing ALP-antibody conjugate and IMP reagent are mixed and incubated at 37°C. In the presence of CK-MB a fluorescein-conjugate: CK-MB: ALP-conjugate complex is formed and captured by the anti fluorescein antibodies on the magnetic particles. The particles are washed and pNPP (para-nitrophenyl phosphate) substrate is added. The ALP in the antibody conjugate reacts with the pNPP to form para-nitrophenoxide and phosphate. Increasing absorbance due to the formation of paranitrophenoxide is monitored at 405 nm and 450 nm. The dose response curve is directly proportional to the concentration of CK-MB in the sample. A quadratic fit through zero is used to construct the dose response curve.
The assay has a range of 0 to 300 ng/ml and calibrators are provided with values of 0, 5, 10, 30, 100 and 300 ng/ml.
All the results reported herein were obtained by using a quadratic fit through zero algorithm to construct the standard curve.

ASSAY PERFORMANCE: COMPARISON OF RESULTS WITH SERUM AND PLASMA SAMPLES

The comparison of Immuno 1 CK-MB results with serum and plasma samples was made with specimens submitted for CK-MB analysis from patients with suspected acute myocardial infarction. Only specimens from patients who had both serum and lithium heparin plasma samples drawn at the same time were used. Both serum and plasma samples from a patient were tested together. The study was conducted at three independent sites. The results of the regression analysis is shown in Table 1 below.

Table 1
COMPARISON OF IMMUNO 1 CK-MB RESULTS FOR SERUM AND PLASMA.

Regression equation is
Plasma CK-MB = Serum CK-MB(Slope) + Intercept

	Number of samples	Range of results (ng/mL)	Slope	Intercept ng/mL	r	Sy.x ng/mL
Site 1	45	0.34 to 24.75	1 06	0.23	0.984	1.30
Site 2	60	0.30 to 334.20	1 06	0.70	0.999	2.45
Site 3	55	0.64 to 202.36	1.06	0.56	0.995	4.69

PLASMA SAMPLE HANDLING

A total of 10 volunteers donated about 30 mL of blood each using heparinized vacutainer tubes. The plasma was separated and 10 mL was spiked with about 5 ng/mL of CK-MB. The plasma was then divided into 1mL aliquots. One was assayed immediately after preparation and the others were stored refrigerated or froxen at -20°C. At the intervals indicated in Table 3 below an aliquot was tested on the Immuno 1. The results indicate that a sample can be stored refrigerated for at least 7 days without change in the Immuno 1 result or for one month frozen at -20°C. The results for the first 8 days were obtained using a calibration curve stored at the start of the study. The system was recalibrated for the results with frozen samples after one month storage. The results for the samples are the means of three replicates

Table 2
IMMUNO 1 CK-MB RESULTS FOR PLASMA SAMPLES STORED UNDER DIFFERENT CONDITIONS.

	Room temperature	Refrigerated at 2 - 8°C					Frozen at -20°C
Sample	Immediate	8 hours	24 hours	48 hours	7 days	8 days	1 month
1	5.6	5.8	5.5	5.5	5.8	5.4	5.6
2	6.7	7.0	6.7	6.5	6.6	6.6	6.3
3	5.2	5.3	5.3	5.3	5.2	5.3	5.2
4	4.4	4.6	4.5	4.5	4.3	4.5	4.2
5	6.1	6.4	6.2	6.1	6.1	6.0	5.8
6	5.9	6.2	6.1	6.0	6.0	6.0	5.7
7	5.1	5.4	5.2	5.3	5.2	5.2	4.8
8	4.2	4.5	4.3	4.3	4.2	4.3	3.9
9	4.4	4.6	4.5	4.6	4.5	4.5	4.3
10	6.8	6.9	7.4	8.2	7.4	7.0	7.3

IMPRECISION: COMPARISON OF RESULTS WITH SERUM AND PLASMA SAMPLES

Results obtained in the first 8 days of the sample handling study described above were used to calculate the imprecision with plasma samples. This is shown in Table 3. Serum controls had been measured in each run and are included for comparison. There is no significant difference between the imprecision obtained for serum or plasma.

Table 3 : IMPRECISION WITH PLASMA SAMPLES

Sample	Mean (ng/mL)	Within-run SD (ng/mL)	Within-run CV (%)	Total SD (ng/mL)	Total CV (%)
Plasma 1	5.61	0.14	2.6	0.18	3.2
Plasma 2	6.65	0.14	2.1	0.18	2.8
Plasma 3	5.28	0.15	2.9	0.14	2.6
Plasma 4	4.48	0.09	2.1	0.11	2.4
Plasma 5	6.14	0.12	1.9	0.13	2.1
Plasma 6	6.03	0.08	1.4	0.10	1.7
Plasma 7	5.23	0.12	2.3	0.12	2.3
Plasma 8	4.27	0.09	2.1	0.10	2.3
Plasma 9	4.51	0.10	2.3	0.11	2.4
Plasma 10	7.27	0.17	2.3	0.54	7.4
Serum Control 1	3.31	0.11	3.3	0.12	3.7
Serum Control 2	12.75	0.15	1.2	0.28	1.2

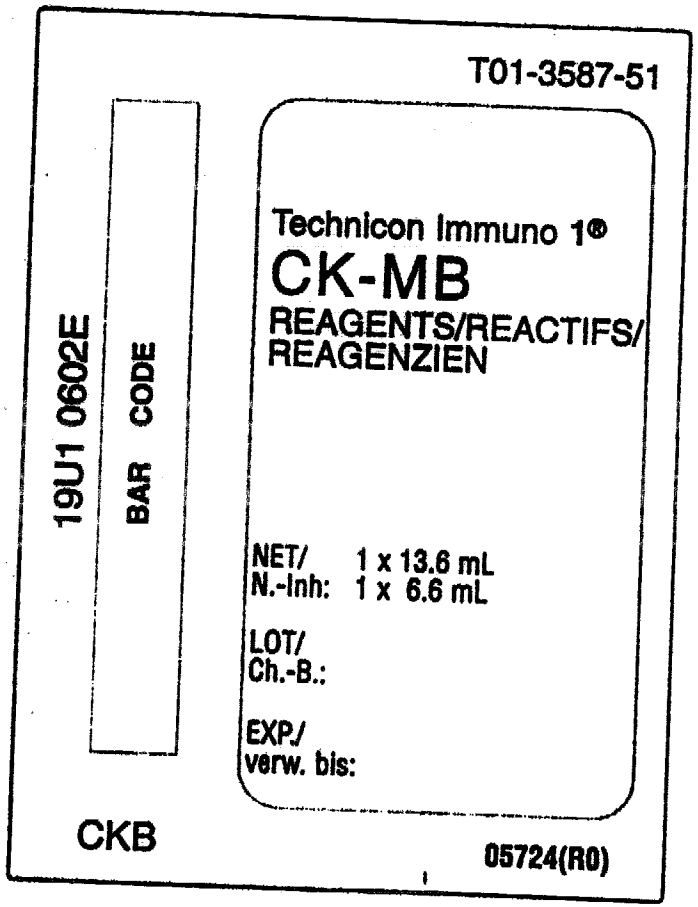
SAMPLE HANDLING INSTRUCTIONS

Serum and plasma (heparin) samples may be used. Samples may be stored for one week at 2 to 8°C or for one month at -20°C. Frozen samples should be thawed at room temperature and mixed thoroughly before use. Thawed samples should not be refrozen. For optimal results the sample must be free of particulate matter.

Increased clotting times may occur with samples from patients receiving anticoagulant or thrombolytic therapy. In those cases only plasma samples should be used! Serum samples containing anticoagulants or thrombolytic agents may yield false positive results on a random basis. Plasma samples collected from these patients using lithium heparin do not appear to exhibit similar problems.

Ensure that clot formation is complete before centrifugation of serum samples.
Fibrin may appear in stored plasma samples. **All plasma samples should be centrifuged or filtered before analysis to ensure removal of particulate matter.**

IN VITRO DIAGNOSTIC PRODUCT LABEL SPECIFICATION



PROOF IS AT: **200%**
SIZE: **1.875" x 2.5"**

COLORS

BLACK

REPRODUCTION TYPE SIZE:
BODY COPY: 8 pt
DISCRETE NO: 6 pt

PRELIMINARY

11/18/94

DRAFT

Attachment 4