510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k102706

B. Purpose for Submission:

New device

C. Measurand:

Creatine phosphokinase/creatine kinase or isoenzymes test system

D. Type of Test:

Quantitative immunoassay

E. Applicant:

SENTINEL CH. SpA

F. Proprietary and Established Names:

CKMB UDR Assay

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
JHS	Class II	21 CFR 862.1215	Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See indication for use below.

2. Indication(s) for use:

The CKMB UDR assay is an *in vitro* diagnostic test used for the kinetic quantitative determination on Unicel DxC 600 System of the CK-MB isoenzyme activity of creatine kinase in serum and Li-heparin plasma by inhibition method. The assay is intended for professional use only. Creatine Kinase (CK) catalyses the reversible phosphorylation of creatine by ATP. CK is a dimer composed of two subunits which form three active isoenzymes: BB (CK-1), MB (CK-2), MM (CK-3). CK-BB isoenzyme only rarely appears in serum.

Elevated CK values are due to muscular damages and associated pathologies. CK determination, usually performed with CK2 (also called CK-MB), is used for the diagnosis and follow-up of AMI (acute myocardial infarction) and some muscular diseases.

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   Beckman Unicel DxC 600 Chemistry Analyzer

I. Device Description:

An *in vitro* diagnostic test used for the kinetic determination of CK-MB isoenzyme activity of creatine kinase by inhibition method. The estimated number of tests per kit is 268. Each assay kit contains 2 reagents.

- Reagent 1 (44 mL):
  - 100 mM imidazole buffer (pH 6.1)
  - 20 mM glucose
  - 20 mM NAC
  - 10 mM magnesium acetate
  - 2 mM NADP
  - 2mM EDTA-Na2
  - ≥ 4 kU/L hexokinase
  - anti-CK-M antibodies
  - < 0.1% sodium azide
- Reagent 2 (12 mL, ph 9.0):
  - 30 mM creatine phosphate
  - 2 mM ADP
  - 5 mM AMP
  - 10 µM diadenosine pentaphosphate
  - ≥ 2.8 kU/L G6P-DH
  - < 0.1% sodium azide

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Roche CK-MB

2. Predicate 510(k) number(s):

   k003158

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	Quantitative *in vitro* determination of CK-MB	Same
Assay Protocol	UV assay with immunological inhibition of human CK-M isoenzyme	Same
Primary Wavelength	340 nm	Same
Antibody	Mouse anti-human CK-M monoclonal	Same
Reference Range	9 – 25 U/L	Same
Reagent Format	Two liquid reagents – ready to use	Same

Differences		
Item	Device	Predicate
Sample Type	Human serum and Li-heparin plasma	Human serum and EDTA plasma
Measuring Range	9 – 600 U/L	5 – 2300 U/L
Instrument	Beckman Unicel DxC 600	Roche/Hitachi Modular P800

K. Standard/Guidance Document Referenced (if applicable):

- CLSI EP05-A2: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition
- CLSI EP06-A: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline
- CLSI Guideline EP7-A2: Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition
- CLSI Document EP9-A2: Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition

- CLSI Document EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline

L. Test Principle:

Anti CK-M mouse monoclonal antibodies in reagent 1 inhibit the CK-M subunit in the sample without affecting the CK-B subunits. The CK-B activity is determined by the CK-NAC method and corresponds to half of the CK-MB activity, where creatine kinase catalyzes the conversion of creatine phosphate and ADP to creatine and ATP. ATP then phosphorylates glucose to glucose-6-phosphate in the presence of hexokinase. Finally, glucose-6-phosphate is oxidized to 6-phosphogluconate, reducing $NADP^+$ to NADPH in presence of glucose-6-phosphate dehydrogenase. The rate of increase in NADPH formed is proportional to the creatine kinase activity in the specimen and is measured kinetically.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision studies were conducted in accordance with CLSI document EP05-A2 and by following the sponsor's pre-approved internal protocols and acceptance criteria. Within run and total imprecision were calculated using 2 levels of commercial QC material, 8 levels of human serum pools, and 1 level of spiked human serum pool. Samples were analyzed twice a day in duplicate, for 10 days, using the Unicel DxC 600 instrument. All results met the sponsor's acceptance criterion of CV ≤ 7.5%.

Sample	N	Mean (U/L)	Within Run		Total Imprecision	
			SD (U/L)	CV%	SD (U/L)	CV%
Low Pool 1	40	19.0	0.49	2.6	0.49	2.6
Low Pool 2	40	17.5	0.30	1.7	0.52	3.0
Low Pool 3	40	18.0	0.42	2.3	0.42	2.3
Low Pool 4	40	10.7	0.45	4.2	0.42	4.2
Level 1 – QC	40	29.9	0.87	2.9	0.81	2.9
Level 2 – QC	40	117.3	0.98	0.8	0.33	1.1
Level 3 – Serum Pool	40	31.2	0.98	3.1	0.98	3.1
Level 4 – Serum Pool	40	33.4	1.21	3.6	0.21	3.6
Level 5 – Spiked	40	584.1	3.76	0.6	5.11	0.9

Serum Pool						
Level 6 – Serum Pool	40	20.1	0.61	3.0	0.61	3.0
Level 7 – Serum Pool	40	25.4	0.50	2.0	0.50	2.0

b. *Linearity/assay reportable range:*

Linearity studies were conducted following the sponsor's internal protocol and acceptance criteria and in accordance with CLSI document EP06-A. Two pools were used to assess the linearity of the assay. Pool 1 was human serum spiked with a concentrated solution of CK-MB to obtain a high activity of 650.19 U/L. Pool 2 was a human serum pool. The two pools were diluted to obtain samples with values ranging from 5.73 to 650.19 U/L. Linearity across the entire measuring range showed a linear regression analysis that resulted in a slope of 1.00 and a y-intercept of 0.00 (r^2=1.00). All data met the sponsor's pre-determined acceptance criteria. The studies support the sponsor's claimed measuring range of 9 to 600 U/L.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Traceability

Traceability of the results of both methods against the IFCC Methods was verified with the Certified Reference Material ERMDA455/ IFCC, based on preapproved protocol and pre-approved acceptance criteria. The sponsor's acceptance criteria are that the percent recovery against the Certified Target value of the ERM-DA455/IFCC must be from 92% to 108%, which is equivalent to the uncertainty of the ERM-DA455/IFCC certified value (± 4 U/L) by factor 2.

Expected Values

A reagent handling study was performed to asses the risk in case of improper handling, by switching reagent 1 with reagent 2 in the Unicel cartridge compartments. Two samples were tested with 5 lots of reagents under these conditions and the result in every case was an error message on the instrument, demonstrating that improper reagent handling will prevent the release of reportable results.

d. *Detection limit:*

A limit of blank (LoB) study was conducted where analyte-free saline was assayed 20 times each on three separate analytical runs using the Unicel DxC 600 analyzers. LoB, or analytical sensitivity claim, was calculated as the absolute mean plus 3 standard deviations. The LoB of 2.23 U/L obtained from this study met the sponsor's acceptance criterion of ≤ 3 U/L (the

analytical sensitivity claim of the predicate device).

A limit of quantitation (LOQ) study was conducted by serially diluting a pool of human sera (approximately 30 U/L) and analyzing each dilution in 10 replicates. The mean, SD, CV%, absolute bias, and total error was calculated for each sample. The sponsor's acceptance criterion was a % total error of ≤ 24.1%. The resulting LOQ was 7.4 U/L. The sponsor's claimed measuring range for the assay is 9 – 600 U/L.

e. Analytical specificity:

Common endogenous interfering substances were evaluated in accordance with CLSI Guideline EP7-A2. Human serum samples at approximate CK-MB concentrations of 25 U/L and 45 U/L were spiked with various concentrations of interferents and evaluated in triplicate at each interferent level. Absolute bias was calculated at each concentration level as compared with non-spiked human serum. Acceptance criterion was given as an absolute bias of ±2.4 U/L (±10% at the clinical decision level of 24 U/L). The results for the 25 U/L CK-MB samples and the 45 U/L CK-MB samples are shown in the table below. Interference from hemoglobin was not found at 40 mg/dL, however significant interference was found at 50 mg/dL hemoglobin. The sponsor has notified users in their package insert to not use hemolyzed samples as hemoglobin > 40 mg/dL interferes with the test.

Interfering Substance	**Concentration (mg/dL)**	**Absolute Bias (mg/dL) - 25U/L**	**Absolute Bias (mg/dL) - 45U/L**
Bilirubin (conjugated)	66.0	-0.95	2.40
Bilirubin (unconjugated)	66.0	1.02	1.30
Hemoglobin	50.0	INTERFERE	INTERFERE
Lipids	1000.0	-1.18	-2.10
Pyruvate	3.0	-0.24	-2.30
Ascorbic Acid	60.0	-0.87	-2.40
Total Protein	14.0	-1.80	-2.40

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

In accordance with EP09-A2, a set of 373 patient human sera was tested for CK-MB using the CKMB UDR assay on the UniCel DxC 600 System and using the Roche CK-MB on Roche/Hitachi Modular P800 Analyzer. Linear

regression analysis and Bland-Altman bias analysis was performed for results comparison. The sponsor's stated acceptance criteria were a linear regression slope between 0.95 and 1.05, and a correlation coefficient (Pearson) r > 0.975. Sample results ranged from 9.0 to 599.2 U/L, and linear regression analysis generated a slope of 0.96 with a y-intercept of 2.16 and a correlation coefficient of 0.998. The Bland-Altman calculated average bias ranged from -10.11 to 11.14 U/L.

b. Matrix comparison:

A matrix comparison study between serum and lithium heparin plasma samples was conducted using serum as reference, in accordance with CLSI Document EP9-A2. The study was performed on 52 matched patient samples. The slope, intercept and correlation coefficient were calculated by linear regression, and a Bland-Altman bias analysis was performed. Acceptance criteria were a slope between 0.95 and 1.05, and a correlation coefficient (Pearson) r > 0.975. The samples ranged from 9 to 582.1 U/L. The resulting equation was y= 0.95x + 0.62 with a correlation coefficient of 1.000.

The compatibility of different collection tubes used for blood sample collection was evaluated using five sample pools assayed in replicates of 5. The absolute bias was calculated for each tube type as compared to the non-anticoagulated serum baseline specimen. Acceptance criterion was given as an absolute bias of ±2.4 U/L (±10% at the clinical decision level of 24 U/L). Both the Lithium Heparin PST (Gel barrier) tube and the SST (Gel barrier) Serum tube generated results within the acceptance criterion when compared to the serum baseline sample results.

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable

b. Clinical specificity:

Not applicable

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

A reference range study was performed using serum samples from 256 apparently healthy subjects (127 male and 127 female). All patients were tested and found to have normal myoglobin results, indicating no apparent heart disease in the reference range study population. Samples were tested in duplicate using the Unicel DxC 600 instrument, and gender-specific reference ranges were determined using the $2.5^{th} – 97.5^{th}$ reference intervals, with a 90% confidence interval. The determined reference ranges were as follows:

Male:	9 – 22.8 U/L
Female:	9 – 27.3 U/L

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.