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

A. 510(k) Number:

k162526

B. Purpose for Submission:

New device

C. Measurand:

Creatine kinase MB subunit

D. Type of Test:

Quantitative, enzymatic assay

E. Applicant:

Roche Diagnostics

F. Proprietary and Established Names:

Creatine Kinase-MB

G. Regulatory Information:

1. Regulation section:

   21 CFR 862.1215, Creatine phosphokinase/creatine kinase or isoenzymes test system

2. Classification:

   Class II

3. Product code:

   JHW, U.V. Method, Cpk Isoenzymes

4. Panel:

   Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See Indications for use below.

2. Indication(s) for use:

   The Creatine Kinase-MB assay is an in vitro test for the quantitative determination of the catalytic activity of creatine kinase MB subunit (CK-MB) in human serum and plasma on Roche/Hitachi cobas c systems.

   Measurements of creatine phosphokinase and its isoenzymes are used in the diagnosis and treatment of myocardial infarction and muscle diseases such as progressive, Duchenne-type muscular dystrophy.

3. Special conditions for use statement(s):

   For prescription use only.

4. Special instrument requirements:

   Studies were performed using the Roche cobas c501 chemistry analyzer.

I. Device Description:

The Creatine Kinase-MB assay consists of two reagents:

- R1 Imidazole buffer: 123 mmol/L, pH 6.5 (37 °C); EDTA: 2.46 mmol/L; Mg2+: 12.3 mmol/L; ADP: 2.46 mmol/L; AMP: 6.14 mmol/L; diadenosine pentaphosphate: 19 µmol/L; NADP (yeast): 2.46 mmol/L; N-acetylcysteine: 24.6 mmol/L; HK (yeast): ≥ 36.7 µkat/L; G6P-DH (E. coli): ≥ 23.4 µkat/L; preservative; stabilizers; additives.
- R2 CAPSO buffer: 20 mmol/L, pH 8.8 (37 °C); glucose: 120 mmol/L; EDTA: 2.46 mmol/L; creatine phosphate: 184 mmol/L; 4 monoclonal anti-CK-M antibodies (mouse), inhibiting capacity: > 99.6 % up to 66.8 µkat/L (4000 U/L) (37 °C) CK-M subunit; preservative; stabilizers; additive.

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	Predicate CK-MB (k003158)	Candidate Device Creatine kinase-MB
Intended Use	In vitro quantitative determination of creatine kinase MB isoenzyme in human serum and plasma.	Same
Sample Type/Matrix	Serum and plasma	Same
Traceability/ Standardization	Traceable to the IFCC CK method	Same

Differences		
Item	Predicate CK-MB (k003158)	Candidate Device Creatine kinase-MB
Reagent Composition	R1 Imidazole buffer and R2 CAPSO buffer.	R1 Imidazole buffer (different HK (yeast) and G6PDH concentrations) and R2 CAPSO buffer. Additives have been added to both buffers.
Reagent On-Board Stability	28 days opened and refrigerated on the analyzer	8 weeks on-board in use and refrigerated on the analyzer
Measuring Range	5 – 2300 U/L (0.08 – 38.4 µkat/L)	10 – 2000 U/L (0.08-33.4 µkat/L)
Detection Limits	LDL = 5 U/L	Limit of Blank = 3 U/L (0.05 µkat/L) Limit of Detection = 3 U/L (0.05 µkat/L) Limit of Quantitation = 10 U/L (0.08 µkat/L)

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

- CLSI EP5-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline.
- CLSI EP6-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline.
- CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline.

L. Test Principle:

Creatine kinase (CK) catalyzes the dephosphorylation of creatine phosphate, generating ATP from ADP. Glucose is then phosphorylated by the ATP formed in the previous reaction to form D-glucose-6-phosphate (G6P), a process catalyzed by hexokinase (HK). Finally, D-glucose-6-phosphate dehydrogenase (G6PDH) catalyzes the oxidation of G6P by NADP to form D-6-phosphogluconate and NADPH. The rate of NADPH formation, determined by measuring the increase in absorbance photometrically, is directly proportional to catalytic CK activity.

Human CK-MB is composed of two subunits, CK-M and CK-B which both have an active site. With the aid of specific antibodies to CK-M, the catalytic activity of CK-M subunits in the sample is inhibited to 99.6 % without affecting the CK-B subunits. The remaining CK-B activity, corresponding to half the CK-MB activity, is determined by the total CK method. As the CK-BB isoenzyme only rarely appears in serum and the catalytic activity of the CK-M and CK-B subunits hardly differ, the catalytic activity of the CK-MB isoenzyme can be calculated from the measured CK-B activity by multiplying the result by 2.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. *Precision/Reproducibility:*

   Precision experiments were performed in accordance with the CLSI Guideline EP5-A3. Five serum samples and 2 control levels were tested using 2 aliquots per run and 2 runs per day for ≥ 21 days on the same cobas c 501 analyzer using 3 reagent lots. Repeatability (within run precision) and intermediate precision (within lab/within device precision incorporating run-to-run and day-to-day precision) were calculated. Precision results for the combined lots are shown below.

   Within-run precision:

Specimen	Mean (U/L)	SD (U/L)	CV (%)
Human Serum 1	17.9	0.4	2.2
Human Serum 2	29.1	0.4	1.2
Human Serum 3	524	2.5	0.5
Human Serum 4	1040	4.9	0.5
Human Serum 5	1826	25	1.3
PreciControl ClinChem Multi 1	41.0	0.3	0.8
PreciControl ClinChem Multi 2	99.2	0.5	0.5

Within-lab precision:

Specimen	Mean (U/L)	SD (U/L)	CV (%)
Human Serum 1	17.8	0.5	2.8
Human Serum 2	29.0	0.6	1.9
Human Serum 3	531	4.4	0.8
Human Serum 4	1040	8.4	0.8
Human Serum 5	1851	42	2.3
PreciControl ClinChem Multi 1	40.2	0.7	1.7
PreciControl ClinChem Multi 2	98.7	1.5	1.5

b. *Linearity/assay reportable range:*

A linearity study was conducted according to CLSI guideline EP6-A. A dilution series was prepared using human sample pools (one serum pool and one plasma pool) with CK-MB concentrations to cover the claimed measuring range. The ranges tested were 0.4 to 2203.6 U/L for serum and 0.7 to 2709.3 U/L for plasma. Dilutions were made using 0.9% NaCl. The dilution series contains 16 concentrations for serum and 18 concentrations for plasma. Samples were measured in triplicate on a cobas c 501 analyzer and data analysis was done separately for each sample. An assessment of linearity was performed using polynomial regression analysis for 1st, 2nd, and 3rd order polynomials. The Creatine Kinase-MB test results were plotted against the expected concentrations. Analysis of the regression coefficients (using a significance level of 5%) showed that the nonlinear coefficients in both the second and third order were significant. The 3rd order was determined to have the best fit (smaller RootMSE) and was used for the calculation of deviation from linearity. The deviation from linearity met the pre-defined acceptance criterion and was within 7%.

The claimed measuring range for CK-MB is 10 to 2000 U/L for serum and plasma.

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

Traceability: This method has been standardized against the IFCC Method for Creatine Kinase with addition of antibodies.

Stability: The reagent shelf life stability claim is 12 months at 2-8°C. The reagent onboard (in use and refrigerated) stability claim is 8 weeks. The protocols were reviewed and found acceptable.

Calibrator: The calibrator used with the CK-MB assay is the previously cleared Roche Diagnostics Calibrator for Automated Systems (k101456).

d. Detection limit:

Limits of detection studies followed the guidelines in CLSI EP17-A2.

The Limit of Blank (LoB) was determined as the 95th percentile of measurements of blank samples. The LoB calculation was performed with one analyte-free sample measured in 10 replicates with 3 reagent lots over 6 runs, 3 days, and on one cobas c 501 analyzer. In total, 60 measurements were obtained per lot. Data analysis is based on determination of the 95th percentile of the 60 measured values.

The Limit of Detection (LoD) was defined as the lowest amount of analyte in a sample detected with a 95% probability. For determination of LoD, five human serum samples with low analyte concentrations (approximately up to 4 times the LoB) were run in duplicate with 3 reagent lots over 6 runs, 3 days, and on one cobas c 501 analyzer. In total, 60 measurements were obtained per lot. LoD was determined using the following equation: $LoD = LoB + 1.653 \times SD_{tot.}$

The Limit of Quantitation (LoQ) is defined as the lowest analyte concentration that can be quantified with a %CV of no more than 20%. For determination of LoQ, a low level sample set was prepared by diluting 5 human serum samples with an analyte free diluent (0.9% NaCl). The low level sample set was tested in 5 replicates per sample on 5 days, one run per day on one cobas c 501 analyzer.

The results of the evaluation of the detection limits for one representative reagent lot and the final claim are in the table below.

	Reagent Lot 2 (U/L)	Claim (U/L)
Limit of Blank	0.3	3
Limit of Detection	1.0	3
Limit of Quantitation	1.9	10

e. Analytical specificity:

Endogenous interference:

The effects of interference by hemoglobin, lipemia (Intralipid), and bilirubin on the CK-MB test system was determined on the cobas c 501 analyzer using pooled human serum samples with 2 CK-MB levels (Level 1: ~18 U/L, Level 2: ~1180 U/L) and spiked with varying levels of interferent. The resulting sample series (10 interferent levels per sample) were tested in triplicate and the mean values were used to calculate % recovery, by comparing the measured concentration to the expected concentration (i.e. CK-MB concentration when no interferent is added). A compound was identified as an interferent if the difference between the spiked test sample and the reference sample was >10%. The results are shown in the table below.

Interferent	No interference up to:	Information in the labeling
Conjugated Bilirubin	Level 1: 76 I Index Level 2: 76 I Index	No significant interference up to an I index of 60 for conjugated and 20 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 60 mg/dL and approximate unconjugated bilirubin concentration: 20 mg/dL).
Unconjugated Bilirubin	Level 1: 28 I Index Level 2: 67 I Index	
Lipemia	Level 1: 753 L Index Level 2: 632 L Index	No significant interference up to an L index of 500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration. Choose diluted sample treatment for automatic rerun.

Hemolysis interferes with the assay. The labeling states; Do not use hemolyzed samples.

Exogenous Interference:

Two serum sample pools containing 2 levels of CK-MB (Level 1: ~22 U/L, Level 2: ~1065 U/L) were divided into aliquots. The concentration of an unspiked aliquot, used as a reference sample, was determined in triplicate on a cobas c501 analyzer. The other sample aliquots were spiked with the respective amount of drug and their CK-MB concentrations determined in triplicate. The means of the triplicate determinations for spiked samples and the reference were compared. A drug was identified as an interferent if the difference between the spiked test sample and the reference sample was >10%.

No interference was observed in the presence of Acetylcysteine (1660 mg/L), Ampicillin-Na (1000 mg/L), Ascorbic acid (300 mg/L), Cyclosporine (5 mg/L), Heparin (5000 U), Levodopa (20 mg/L), Methyldopa +1.5 (20 mg/L), Metronidazole (200 mg/L), Phenylbutazone (400 mg/L), Doxycycline (50 mg/L), Acetylsalicylic Acid (1000 mg/L), Rifampicin (60 mg/L), Acetaminophen (200 mg/L), Ibuprofen (500 mg/L), and Theophyllin (100 mg/L).

The labeling states that no interference was found at therapeutic concentrations using common drug panels.

Interference was observed with Cefoxitin and Cyanokit. The labeling states that Cyanokit (Hydroxocobalamin) and Cefoxitin at therapeutic concentrations interfere with the test.

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

A total of 105 human serum samples with values ranging from 10.1 to 1939 U/L were tested in singlicate with the CK-MB assay on a Roche cobas c 501 analyzer and the predicate device. Four of the 105 samples were spiked with recombinant human CK-MB. The data were evaluated using Passing-Bablok regression analysis. The results are shown below.

y =0.977x + 1.12 U/L, r = 0.968

b. Matrix comparison:

A matrix comparison study was performed by comparing reference serum samples (in serum tubes) with 31 serum samples (in gel separation tubes) or plasma samples (in 30 K2 EDTA tubes, 31 K3 EDTA tubes, or 31 Li Heparin tubes). Samples ranging from 10.1 to 1956 U/L were tested in singlicate on the cobas c 501 analyzer. Regression analysis was performed using the serum data as the reference. The results of these studies are shown below.

Anticoagulant	Regression Analysis	R
Serum vs. Serum Gel Separation	y = 0.996x + 0.804 U/L	1.00
Serum vs. Li-heparin	y = 1.00x – 0.616 U/L	0.999
Serum vs. K2-EDTA	y = 1.00x – 0.717 U/L	0.999
Serum vs. K3-EDTA	y = 0.995x - 0.062 U/L	1.00

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:

For healthy people, according to Klein *et al.*[1] and consensus values[2]: CK-MB < 25 U/L.

The reagents used in the reference interval studies in Klein, *et al.*, are identical to the proposed device. Reference intervals for CK-MB were performed at 6 sites with healthy individuals (202 males and 217 females ranging in age from 20-60 years). Reference intervals were reported as follows: CK-MB (2.5-97.5%) of 8-24 U/L for males and CK-MB (2.5-97.5%) of 6-25 U/L for females.

The labeling states:

Reference intervals strongly depend on the patient group regarded and the specific clinical situation. Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference ranges.

[1]Klein G, Berger A, Bertholf R, et al. Abstract: Multicenter Evaluation of Liquid Reagents for CK, CK-MB and LDH with Determination of Reference Intervals on Hitachi Systems. Clin Chem 2001; 47:Suppl.A30.

[2]Thomas L, Müller M, Schumann G, et al. Consensus of DGKL and VDGH for interim reference intervals on enzymes in serum. J Lab Med 2005; 29(5):301-308.

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.