

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

A. 510(k) Number:

k160570

B. Purpose for Submission:

New device

C. Measurand:

Creatine Kinase

D. Type of Test:

Quantitative

E. Applicant:

Roche Diagnostics Operations (RDO)

F. Proprietary and Established Names:

Creatine Kinase

G. Regulatory Information:

1. Regulation section:

21 CFR § 862.1215

2. Classification:

Class II

3. Product code:

JHS

4. Panel:

Chemistry (75)

H. Intended Use:

1. Intended use(s):

See Indications for use below

2. Indication(s) for use:

Creatine kinase is an in vitro test for the quantitative determination of creatine kinase (CK) in human serum and plasma on Roche/Hitachi cobas c systems. The determination of CK and CK isoenzyme activities is utilized in the diagnosis and monitoring of myocardial infarction and myopathies such as the progressive Duchenne muscular dystrophy.

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

Roche/Hitachi cobas c 501 analyzer

I. Device Description:

The Creatine Kinase assay is a two reagent (R1 and R2) assay for the quantitative determination of creatine kinase (CK) in human serum and plasma on automated clinical chemistry analyzers. Photometrically measured NAPDP formation is directly proportional to CK activity in a human sample.

- R1 contains 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; G6PDH (E. coli): ≥ 23.4 µkat/L; preservative; stabilizers; additives.
- R2 contains CAPSO buffer: 20 mmol/L, pH 8.8 (37 °C); glucose: 120 mmol/L; EDTA: 2.46 mmol/L; creatine phosphate: 184 mmol/L; preservative; stabilizers.

J. Substantial Equivalence Information:

1. Predicate device name(s):

COBAS INTEGRA Creatine Kinase

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

k951595

3. Comparison with predicate:

Similarities		
Item	Candidate Device Creatine Kinase	Predicate COBAS INTEGRA Creatine Kinase (k951595)
Intended use	An in vitro test for the quantitative determination of creatine kinase (CK) in human serum and plasma	Same
Sample types	Non hemolyzed Serum and Plasma: Li-Heparin, K2 and K3 EDTA.	Same
Reagent Shelf Life Stability	12 months at 2 - 8°C	Same
Traceability	Standardized against the IFCC Method for Creatine Kinase	Same
Calibration frequency	After reagent lot change and as required following quality control procedures	Same

Differences		
Item	Candidate Device Creatine Kinase	Predicate COBAS INTEGRA Creatine Kinase (k951595)
Measuring range	7 – 2000 U/L (0.12-33.4 µkat/L)	0 – 2000 U/L (0 - 33.4 µkat/L)
Calibrator	Roche Diagnostics Calibrator for Automated Systems (k101456)	Calibrator (human)

Differences		
Item	Candidate Device Creatine Kinase	Predicate COBAS INTEGRA Creatine Kinase (k951595)
Controls	Precinorm U plus/Precipath U Plus (k042389) Precinorm CK-MB/Precipath CK-MB (k062972) PeciControl ClinChem Multi 1 and 2 (k102016)	Control Serum N (human) Control Serum P (human)
Lower Limits of Measurement	LoB = 7 U/L (0.12 μ kat/L) LoD = 7 U/L (0.12 μ kat/L) LoQ = 7 U/L (0.12 μ kat/L)	LDL = 0 U/L
Reagent On-Board Stability	On-board (refrigerated at 8°C) on the analyzer: 8 weeks	On-board (in use at 8°C): 4 weeks
Sample Stability	Stability in serum: 2 days at 20-25 °C 7 days at 4-8 °C 4 weeks at -20 °C Stability in EDTA or heparin plasma: 2 days at 15-25 °C 7 days at 2-8 °C 4 weeks at (-15-25)°C	CK activity in serum remains stable for 24h at +22 °C or 10 days at +4 °C and -20 °C.
Reagents	2 Reagents; R1 and R2. See composition in description above.	3 Reagents; R1: Buffer in vial A (15.8 mL). R2: Enzyme granulate in vial B (0.5 g for 12.3 mL). R3 = SR: Creatine phosphate granulate in vial C (0.7 g for 5 mL).

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

CLSI/ EP17-A, Protocols for Determination of Limits of Detection and Limits of Quantitation

CLSI EP5-A2, Evaluation of Precision Performance of Clinical Chemistry Devices

CLSI EP6-A Evaluation of the Linearity of Quantitative Measurement

L. Test Principle:

The Creatine Kinase assay is a UV test for the quantitative determination of creatine kinase (CK) in human serum and plasma on Roche/Hitachi cobas c systems. The CK is activated by N-acetylcysteine (NAC). In a primary reaction, the activated CK catalyzes the dephosphorylation of creatine phosphate. In a coupled reaction, catalyzed by hexokinase (HK), glucose is phosphorylated by the ATP formed in the primary reaction to form D-glucose-6-phosphate (G6P). 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 is directly proportional to catalytic CK activity. It is determined by measuring the increase in absorbance at 340nm (main) and 546nm (sub).

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Precision experiments were performed in accordance with CLSI Guideline EP5-A2. Five serum pools and two levels of control were tested using two aliquots per run, two runs per day for 21 days on the same cobas c 501 analyzer and 3 lots of reagent. Repeatability (within run precision) and intermediate precision (within lab precision) were calculated. Precision results are:

Material	n	Mean (U/L)	Repeatability		Within-lab	
			SD(U/L)	%CV	SD(U/L)	%CV
Serum pool 1	84	18.7	0.6	3.0	0.6	3.2
Serum pool 2	84	137	0.8	0.6	1.1	0.8
Serum pool 3	84	477	3.0	0.6	3.1	0.6
Serum pool 4	84	946	5.3	0.6	5.8	0.6
Serum pool 5	84	1816	9.4	0.5	10	0.6
Control level 1	84	154	0.9	0.6	1.7	1.1
Control level 2	84	301	1.3	0.4	2.6	0.9

b. *Linearity/assay reportable range:*

A linearity study was conducted by preparing dilution series using human sample pools (one serum pool and one plasma pool) with creatine kinase concentrations to cover the claimed measuring range. The ranges tested were 5.3 to 2099 U/L for serum and 3.6 to 2181 U/L for plasma. Dilutions were made using 0.9% NaCl. The dilution series contain 18 concentrations for both serum and plasma. Samples were measured in triplicate on a cobas c 501 analyzer and data analysis was done separately for each sample type. Linear regression analysis was done according to EP6-A. The results of the linearity evaluation are:

Sample Type	Linear Regression	R ²
Serum	$y=1.00x-1.137$	0.9996
Plasma	$y=1.00x-2.842$	0.9997

The claimed measuring range for creatine kinase is 7 – 2000 U/L (0.12-33.4 μ kat/L).

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

Traceability: This method has been standardized against the IFCC Method for Creatine Kinase.

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.

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

Controls: The controls used with the Creatine Kinase assay have been previously cleared:

Precinorm U plus/Precipath U Plus (k042389)
Precinorm CK-MB/Precipath CK-MB (k062972)
PreciControl ClinChem Multi 1 and 2 (k102016)

Sample stability assessment: A plasma sample stability study was performed using native plasma samples collected in Li Heparin, K2 EDTA and K3 EDTA and stored at 15 - 25°C for 2 days, at 2 - 8°C for 7 days and frozen at (-15 to -25)°C for 28 days. The samples were tested using the CK assay on the cobas c 501 analyzer and ranged from 82.2 to 139.1 U/L. The % recovery relative to the analyte content of the time zero samples (reference) was calculated from the median of the threefold measurement. The study supported the sample stability claim of EDTA/heparin plasma: 2 days at 15-25 °C, 7 days at 2-8 °C and 4 weeks at (-15 to -25) °C.

d. Detection limit:

The evaluation of the limits of detection followed the recommendations in CLSI EP17-A, Protocols for Determination of Limits of Detection and Limits of Quantitation.

The Limit of Blank (LoB) was defined as the concentration at which there is a 95% probability that a sample is analyte-free. The LoB calculation was performed with one analyte free sample measured with three reagent lots in 10-fold determination in 6 runs, distributed over 3 days, 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 concentration at which there is a 95% probability that a sample contains analyte. For determination of LoD five samples with low-analyte concentration (approximately up to 4 times the LoB) were measured with three reagent lots in two-fold determination in 6 runs, distributed over 3 days, on one cobas c 501 analyzer. In total 60 measurements were obtained per lot. $LoD = LoB + 1.653 \times SD_{tot}$.

The limit of Quantitation (LoQ) is defined as the lowest analyte concentration that can be quantitatively determined with a stated acceptable precision and trueness under stated experimental conditions. 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. LoQ is calculated with the precision at 20% CV results.

The results of the evaluation of the detection limits are in the table below. The label states that the detection limit of the test system is 7 U/L.

	Result (U/L)	Claim (U/L)
Limit of Blank (LoB)	0.3	7
Limit of Detection (LoD)	1.0	7
Limit of Quantitation (LoQ)	3.3	7

e. Analytical specificity:

Endogenous Interference:

The effects of endogenous interference by hemoglobin, lipemia (Intralipid), and bilirubin on the Creatine Kinase test system was performed on the cobas c 501 analyzer using 2 CK concentrations (Level 1 approximately 120 U/L and Level 2 approximately 1400 U/L of pooled human serum samples spiked with varying levels of interferent. The resulting sample series (10 dilution steps per sample) were tested

in triplicate and then the measured concentration was compared to the expected concentration (which is the CK concentration when no interferent was added). The results are:

Interferent	No interference up to	Information in the labeling
Hemolysis	Level 1: 103 H Index Level 2: 130 H Index	No significant interference up to an H index of 100 (approximate hemoglobin concentration: 100 mg/dL)
Lipemia	Level 1: 1356 L Index Level 2: 1143 L Index	No significant interference up to an L index of 1000 There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration. Highly lipemic specimens (L index > 1000) may cause high absorbance flagging. Choose diluted sample treatment for automatic rerun.
Unconjugated Bilirubin	Level 1: 67 I Index Level 2: 67 I Index	No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 60 mg/dL).
Conjugated Bilirubin	Level 1: 68 I Index Level 2: 76 I Index	

Exogenous Interference:

Two sample pools containing a low and high concentration (low is approximately 120 U/L and high is approximately 1400 U/L) of CK were used. These sample pools were divided into an appropriate number of aliquots. One aliquot was not spiked with the drugs and it was used as the reference sample for CK concentration. The other sample aliquots, with either the high or low CK concentrations, were spiked with the respective amount of drug. The CK concentration of the spiked aliquots were determined in triplicate on a cobas c 501 analyzer and compared to the CK concentration determined for the reference aliquot (mean of n=3). The sponsor defined no significant interference as < 10 % difference from the reference sample.

No interference was found when tested using Acetylcysteine (1660 mg/L), Ampicillin-Na (1000 mg/L), Ascorbic acid (300 mg/L), Cyclosporine (5 mg/L), Cefoxitin (250 mg/L), Heparin (5000 U), Intralipid (10,000), Levodopa (4 mg/L), Methyl dopa +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 Theophylline (100 mg/L). The labeling states that no interference was found at therapeutic concentrations using common drug panels.

Interference was found with Cyanokit. The labeling states that Cyanokit (Hydroxocobalamin) at therapeutic concentrations interferes with the test.

f. *Assay cut-off:*

Not applicable.

2. Comparison studies:

a. *Method comparison with predicate device:*

A total of 132 human serum samples with values ranging from 7.59 to 1946 U/L were tested in the method comparison study. The samples were tested in singlicate with the CK assay on the cobas c 501 analyzer and the predicate device. Nine of the 132 samples were spiked with human recombinant CK MB. The data were evaluated using Passing Bablok regression analysis. The results of the analysis are:

$$y = 1.021x + 5.88 \text{ U/L} \quad r = 0.999$$

b. *Matrix comparison:*

A matrix comparison study was performed using samples drawn into serum and different types of plasma collection tubes (K2 EDTA, K3 EDTA, Li Heparin, and Gel Separation). For each of the four tube types, 36 samples ranging from 8 to 1953 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 the studies are:

Anticoagulant	Regression Analysis	r
Serum vs. Serum Gel Separation	$y = 0.998x - 0.168$	0.999
Serum vs. Li-heparin	$y = 0.998x - 1.282$	0.999
Serum vs. K2-EDTA	$y = 0.991x - 1.422$	0.998
Serum vs. K3-EDTA	$y = 0.975x - 0.495$	0.999

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:

Reference intervals for CK depend on the patient group 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.

For healthy people, according to Klein et al. ¹

CK	U/L	μkat/L
Men	39-308	0.65-5.14
Women	26-192	0.43-3.21

The reference values according to Klein et al. are based on the 95th percentile of a group of healthy persons (202 men and 217 women) not involved in high-intensity athletic activities and were established on Roche/Hitachi analyzers using CK reagent.

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

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.