Creatine Kinase (CK) - Serum, Plasma
Beckman UniCel DxC Systems

Technical Procedure 3118

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For *In Vitro* Diagnostic Use Only

Principle

Intended Use

CK reagent, when used in conjunction with UniCel® DxC 800 System(s), is intended for the quantitative determination of creatine kinase activity in human serum or plasma.

Clinical Significance

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

Methodology

CK reagent is used to measure the CK activity by an enzymatic rate method.(1,2,3,4) In the reaction creatine kinase catalyzes the transfer of a phosphate group from the creatine phosphate substrate to adenosine diphosphate (ADP). The subsequent formation of adenosine triphosphate (ATP) is measured through the use of two coupled reactions catalyzed by hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6PDH) which results in the production of reduced β-nicotinamide adenine dinucleotide phosphate (NADPH) from β-nicotinamide adenine dinucleotide phosphate (NADP). The CK assay contains the activator monothioglycerol.

The SYNCHRON® System(s) automatically proportions the appropriate sample and reagent volumes into the cuvette. The ratio used is one part sample to 20 parts reagent. The system monitors the change in absorbance at 340 nanometers. This change in absorbance is directly proportional to the activity of CK in the sample and is used by the System to calculate and express CK activity.

Chemical Reaction Scheme

$$\text{Creatine phosphate + ADP} \xrightarrow{\text{CK}} \text{Creatine + ATP}$$

$$\text{ATP + glucose} \xrightarrow{\text{HK}} \text{Glucose-6-phosphate + ADP}$$

$$\text{Glucose-6-phosphate + NADP}^{+} \xrightarrow{\text{G6PDH}} \text{6-Phosphogluconate + NADPH}^{+} + \text{H}^{+}$$

ADP= adenosine diphosphate
ATP= adenosine triphosphate
HK= Hexokinase
G6PDH= glucose-6-phosphate dehydrogenase
NADP= β-nicotinamide adenine dinucleotide phosphate
NADPH= reduced β-nicotinamide adenine dinucleotide phosphate

Specimen

Acceptable Sample Containers

13 x 75 PST, SST and Red Top BD tubes
PST, SST and Red Top BD microtainers

Type of Specimen

Biological fluid samples should be collected in the same manner routinely used for any laboratory test.(5) Freshly drawn serum or plasma are the preferred specimens. Acceptable anticoagulants are listed in *Procedural Notes* section of this chemistry information sheet. Whole blood or urine are not recommended for use as a sample.

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Specimen Storage and Stability

1. Tubes of blood are to be kept closed at all times and in a vertical position. It is recommended that the serum or plasma should be physically separated from contact with cells within two hours from the time of collection.(6)
2. Stability of CK activity in sera is not well defined, but is generally poor. Specimens should be assayed as soon after collection as possible since activity loss may occur after specimens have been stored for 4 hours at room temperature, 8 to 12 hours refrigerated or 2 to 3 days when frozen.(6)

Sample Volume

The optimum volume, when using a 0.5 mL sample cup, is 0.3 mL of sample. For optimum primary sample tube volumes and minimum volumes, refer to the *Primary Tube Sample Template*.

Criteria for Unacceptable Specimens

Refer to the *Procedural Notes* section of this chemistry information sheet for information on unacceptable specimens.

Reagents

Contents

Each kit contains the following items: **Kit Reorder # 476836**

Two CK Reagent Cartridges (2 x 400 tests and two bottles of CK [A-reagent])

Volumes per Test

Sample Volume	13 µL
ORDAC Sample Volume	3 µL
Total Reagent Volume	260 µL
Cartridge Volumes	
A	238 µL
B	22 µL
C	-----

Reactive Ingredients

Reagent Constituents	
Creatine phosphate	53 mmol/L
Glucose	18 mmol/L
ADP	2.9 mmol/L
NAD^+	2.4 mmol/L
Hexokinase	>11 KIU/L
Glucose-6-phosphate dehydrogenase	>3.8 KIU/L

Also non-reactive chemicals necessary for optimal system performance.

Avoid skin contact with reagent. Use water to wash reagent from skin.

Materials Needed But Not Supplied With Reagent Kit

At least two levels of control material
Saline

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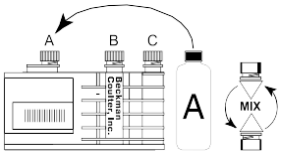
Reagent Preparation

Transfer all the contents of one bottle CK (A-reagent) into the largest reagent compartment (A). Replace cartridge caps and gently invert the cartridge several times to ensure adequate mixing. Before loading, remove all bubbles from reagent compartments.

Date and initial cartridge and document in reagent log before loading each new cartridge.



Acceptable Reagent Performance

The acceptability of this reagent is determined by successful calibration and by ensuring that quality control results are within acceptance criteria, as defined in the Clinical Chemistry Quality Control Procedure #3000.T.

Reagent Storage and Stability

CK reagent, when stored unopened at +2°C to +8°C, will remain stable until the expiration date printed on the cartridge label. Once prepared, the reagent cartridge is stable for 30 days at +2°C to +8°C unless the expiration date is exceeded. **DO NOT FREEZE**.

Equipment

This test is performed on the Beckman UniCel DxC 800 systems; Beckman-Coulter, Brea, California. For technical assistance, call the Beckman-Coulter hotline: 1-800-854-3633.

Refer to the UniCel DxC 800 systems *Reference Manual* for detailed instructions.

Calibration

Calibrator Required

Calibration is not required.

Traceability

This measurand (CK) is traceable to the manufacturer's selected Measurement Procedure as described in the Methodology section.

Quality Control

At least two levels of control material should be analyzed each shift. In addition, these controls should be run with each new calibration, with each new reagent cartridge, and after specific maintenance or troubleshooting procedures as detailed in the UniCel DxC 800 System *Instructions For Use* manual. More frequent use of controls or the use of additional controls is left to the discretion of the user based on workload and workflow.

The following controls should be used in accordance with the package instructions for use inserts. Copies of these inserts can be found in the *Control IFUs* folder on the S drive (*S:\APS\ClinLab\PoliciesandProcedures\1000-8999CLINICALPATHOLOGY\3000-3999Chemistry\3000-3499AutomatedChemistry\ControlIFUs*). Quality control results should be evaluated and handled with respect to the Clinical Chemistry Quality Control Procedure #3000.T. Controls are compiled statistically in the LIS and reagent lot changes are documented on DxC Reagent Log sheets.

Quality Control Material

Control	Storage
MAS ChemTrak 1	+2°C to +8°C*
MAS ChemTrak 3	+2°C to +8°°C*

*Controls are received frozen and stored at –15°C to –25°C.
Bottles of controls in use are thawed and stored at +2°C to +8°C and are good for 14 days.

Testing Procedure

1. If necessary prepare reagent as defined in the Reagent Preparation section of this chemistry information sheet and load the reagent onto the system.
2. Program samples and controls for analysis.
3. After loading samples and controls onto the system, follow the protocols for system operation.

For detailed testing procedures, refer to the UniCel DxC 800 System *Instructions For Use* (IFU) manual.

Calculations

UniCel DxC Systems perform all calculations internally to produce the final reported result. The system will calculate the final result for sample dilutions made by the operator when the dilution factor is entered into the system during sample programming.

If the dilution was programmed in Remisol, the final calculated result from a dilution will not be calculated by the UniCel DxC system but by Remisol.

Reporting Results

Equivalency between the SYNCHRON LX and UniCel DxC 800 Systems has been established. Chemistry results between these systems are in agreement and data from representative systems may be shown.

Reference Intervals

The following reference intervals were taken from literature and a study performed on SYNCHRON Systems.(9,10)

Reference Intervals

Intervals	Sample Type	Conventional Units	S.I. Units
Literature	Serum or Plasma (Male)	38 - 174 IU/L	0.65 - 2.96 µkat/L
	Serum or Plasma (Female)	26 - 140 IU/L	0.46 - 2.38 µkat/L
SYNCHRON	Serum or Plasma (Male)	49 - 397 IU/L	0.83 - 6.75 µkat/L
	Serum or Plasma (Female)	38 - 234 IU/L	0.65 - 3.98µkat/L
UCDMC	Serum or Plasma	22 - 250 IU/L	0.37 - 4.25 µkat/L

Refer to References (6,7,8) for guidelines on establishing laboratory-specific reference intervals

Procedural Notes

Anticoagulant Test Results

1. If plasma is the sample of choice, the following anticoagulants were found to be compatible with this method based on a study of 20 healthy volunteers:

Acceptable Anticoagulants[a]

Anticoagulant	Level Tested for In Vitro Interference	Average Plasma-Serum Bias[a] (IU/L)
Ammonium Heparin	29 Units/mL	NSI[b]
Lithium Heparin	29 Units/mL	NSI
Sodium Heparin	29 Units/mL	NSI

[a] Bias is based on worst case instead of average.

[b] NSI = No Significant Interference (within ±10.0 IU/L or 7%).

2. The following anticoagulants were found to be incompatible with this method:

Incompatible Anticoagulants

Anticoagulant	Level Tested for In Vitro Interference	Average Plasma-Serum Bias[a] (IU/L)
Potassium Oxalate/ Sodium Fluoride	4.0 / 5.0 mg/mL	-80.0
Sodium Citrate	6.6 mg/mL	-97.0

[a] Bias is based on worst case instead of average. Plus (+) or minus (-) signs in this column signify positive or negative bias.

Limitations

None identified

Interferences

1. The following substances were tested for interference with this methodology:

Interferences[a]

Substance	Source	Level Tested	Observed Effect[b]
Bilirubin (unconjugated)	Bovine	30 mg/dL	NSI[c]
Hemoglobin	RBC Hemolysate	50 mg/dL	+12 IU/L
Lipemia	Intralipid[d]	500 mg/dL	NSI
Adenylate Kinase	NA[e]	100 U/L	+8 IU/L

[a] Data shown was collected using SYNCHRON CX Systems. Equivalency between SYNCHRON LX Systems has been established by Deming regression analysis to SYNCHRON CX Systems.

[b] Plus (+) or minus (-) signs in this column signify positive or negative interference.

[c] NSI = No Significant Interference (within ± 6 IU/L or 7%).

[d] Intralipid is a registered trademark of KabiVitrum, Inc., Clayton, NC 27250.

[e] NA = Not applicable

2. Refer to References (11,12,13) for other interferences caused by drugs, disease and preanalytical variables.

Performance Characteristics

Analytical Measurement Range

The SYNCHRON® System(s) method for the determination of alkaline phosphatase provides the following analytical ranges:

Analytical measurement Range (AMR)

Sample Type	Conventional Units	S.I. Units
Serum/Plasma/Fluid	5 - 1200 IU/L	0.1 - 20.0 µkat/L
Serum/Plasma/Fluid (ORDAC)[a]	860 - 4100 IU/L	14.3 - 68.3 µkat/L

[a] Overrange Detection and Correction. Refer to the UniCel DxC 800 System Instructions For Use (IFU) manual for more details on this function.

Clinical Reportable Range:

Clinical Reportable Range (CRR)

Sample Type	Conventional Units	S.I. Units
Serum/Plasma/Fluid	5 - diluted result IU/L (dilute no more than x201)	0.1 - diluted result µkat/L (dilute no more than x201)

Samples with concentrations below the AMR and CRR (5 IU/L) will be reported as "**< 5 IU/L**".
Samples with concentrations greater than the AMR should be diluted with saline and reanalyzed.
If a numerical result cannot obtained with a x201 dilution, result as **>240,000 IU/L**.
If the dilution was programmed in the DataLink, the final calculated result from a dilution will not be calculated by the UniCel DxC system but by the DataLink.

Sensitivity

Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for CK determination is 5 IU/L (0.08 µkat/L).

Equivalency

Equivalency was assessed by Deming regression analysis of patient samples to accepted clinical methods and documented by Beckman.

Serum or Plasma (in the range of 10 to 1126 IU/L):

Y (SYNCHRON LX Systems)	= 1.025X + 0.39
N	= 80
MEAN (SYNCHRON LX Systems)	= 286.5
MEAN (SYNCHRON CX7 Delta	= 279.1
CORRELATION COEFFICIENT (r)	= 0.9991

Refer to References (14) for guidelines on performing equivalency testing.

Equivalency assessed by Deming regression analysis of patient samples to accepted clinical methods as determined at UCDMC.

Serum or Plasma (in the range of 15 to 909 IU/L):

Y (UniCel DxC800-4118)	= 0.991X + 0.1
N	= 23
MEAN (UniCel DxC800-4118)	= 313.3
MEAN (UniCel DxC800-1805)	= 316.0
CORRELATION COEFFICIENT (r)	= 0.9997

Serum or Plasma (in the range of 15 to 909 IU/L):

Y (UniCel DxC800-4427)	= 1.010X - 1.7
N	= 23
MEAN (UniCel DxC800-4427)	= 317.4
MEAN (UniCel DxC800-1805)	= 316.0
CORRELATION COEFFICIENT (r)	= 0.9998

Serum or Plasma (in the range of 15 to 909 IU/L):

Y (UniCel DxC800-4449)	= 1.004X - 0.8
N	= 23
MEAN (UniCel DxC800-4449)	= 316.3
MEAN (UniCel DxC800-1805)	= 316.0
CORRELATION COEFFICIENT (r)	= 0.9998

Serum or Plasma (in the range of 8 to 898 IU/L):

Y (UniCel DxC800-4427)	= 1.019X - 1.9
N	= 23
MEAN (UniCel DxC800-4427)	= 317.4
MEAN (UniCel DxC800-4118)	= 313.3
CORRELATION COEFFICIENT (r)	= 0.9998

Serum or Plasma (in the range of 8 to 898 IU/L):

Y (UniCel DxC800-4449)	= 1.013X - 1.0
N	= 23
MEAN (UniCel DxC800-4449)	= 316.3
MEAN (UniCel DxC800-4118)	= 313.3
CORRELATION COEFFICIENT (r)	= 0.9998

Serum or Plasma (in the range of 9 to 916 IU/L):

Y (UniCel DxC800-4449)	= 0.994X + 0.9
N	= 23
MEAN (UniCel DxC800-4449)	= 316.3
MEAN (UniCel DxC800-4427)	= 317.4
CORRELATION COEFFICIENT (r)	= 0.9999

Precision

A properly operating SYNCHRON® System(s) should exhibit precision values less than or equal to the following:
As determined by Beckman

Precision Values

Type of Precision	Sample Type	1 SD		Changeover Value[a]		%CV
			µkat/L	IU/L	µkat/L	
Within-run	Serum/Plasma	5.0	0.08	142.9	2.29	3.5
	Serum/Plasma (ORDAC)	NA[b]	NA	NA	NA	10.0
Total	Serum/Plasma	7.5	0.12	142.9	2.29	5.3
	Serum/Plasma (ORDAC)	NA	NA	NA	NA	15.0

[a] When the mean of the test precision data is less than or equal to the changeover value, compare the test SD to the SD guideline given above to determine the acceptability of the precision testing. When the mean of the test precision data is greater than the changeover value, compare the test % CV to the guideline given above to determine acceptability. Changeover value = (SD guideline/CV guideline) x 100.

[b] NA = Not applicable.

Precision established at UCDMC

Type of Precision	Sample Type	n	Mean (IU/L)	1 SD (U/L)	%CV
DxC800-4118 Within-run	SYNCHRON 1	20	56.9	0.9	1.5
	SYNCHRON 3	20	697.4	2.5	0.4
DxC800-4427 Within-run	SYNCHRON 1	20	56.9	1.2	2.1
	SYNCHRON 3	20	719.6	5.4	0.7
DxC800-4449 Within-run	SYNCHRON 1	20	57.6	0.7	1.2
	SYNCHRON 3	20	709.3	3.9	0.5

Type of Imprecision	Sample Type	n	Mean (IU/L)	SD	%CV
DxC800-4118 Day to Day	MAS ChemTrak 1	374	113	1.7	1.5
	MAS ChemTrak 3	371	635	11.8	1.9
DxC800-4427 Day to Day	MAS ChemTrak 1	375	115	2.3	2.0
	MAS ChemTrak 3	372	648	12.9	2.0
DxC800-4449 Day to Day	MAS ChemTrak 1	384	115	1.5	1.3
	MAS ChemTrak 3	388	646	11.6	1.8

Comparative performance

Comparative performance data for the SYNCHRON LXSystem evaluated using the NCCLS Proposed Guideline EP5-T2 appears in the table below.(15)

CLSI/NCCLS EP5-T2 Precision Estimate Method

Type of Imprecision	Sample Type	No. Systems	No. Data Points[a]	Test Mean Value (IU/L)	EP5-T2 Calculated Point Estimates	
					SD	%CV
Within-run	Serum Control 1	1	80	52.3	1.6	3.0
	Serum Control 2	1	80	630.0	4.8	0.8
	Serum Human Pool	1	80	258.2	2.9	1.1
Total	Serum Control 1	1	80	52.3	1.9	3.7
	Serum Control 2	1	80	630.0	5.7	0.9
	Serum Human Pool	1	80	258.2	3.4	1.3

[a] The point estimate is based on the data from one system, run for twenty days, two runs per day, two observations per run on an instrument operated and maintained according to the manufacturer's instructions.

NOTICE

These degrees of precision and equivalency were obtained in typical testing procedures on a SYNCHRON LX® System and are not intended to represent the performance specifications for this reagent.

Additional Information

For more detailed information on UniCel DxC Systems, refer to the *Instructions for Use* and *Reference* manual.

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