BQ Kits
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Creatinine Liquid Reagents Assay

Catalog Number: BQ 072A-EAKP

Intended Use

This assay kit is intended for the quantitative determination of creatinine in serum and urine. Creatinine measurements are used in the diagnosis and treatment of renal diseases, in monitoring renal dialysis, and as a calculation basis for measuring other urine analytes. For *in vitro* diagnostic use only.

Clinical Significance

Creatinine is a chemical waste molecule that is generated from muscle metabolism. Creatinine is produced from creatine, a molecule of major importance for energy production in muscles. Approximately 2% of the body's creatine is converted to creatinine every day. Creatinine is transported through the bloodstream to the kidneys. The kidneys filter out most of the creatinine and dispose of it in the urine.

The kidneys maintain the blood creatinine in a normal range. Creatinine has been found to be a fairly reliable indicator of kidney function. As the kidneys become impaired the creatinine level in the blood will rise. Abnormally high levels of creatinine thus warn of possible malfunction or failure of the kidneys, sometimes even before a patient reports any symptoms. It is for this reason that standard blood and urine tests routinely check the amount of creatinine in the blood. [1-2]

Assay Principle

The BQ Kits Creatinine liquid reagents assay is a quick, easy to use enzymatic procedure applicable to routine laboratory instrumentation. Enzymatic methodology is a better clinical choice for the accurate measurement of creatinine, especially for neonates, pediatrics, and hematology units.[3]

The enzymatic assay for creatinine involves a series of coupled enzymatic reactions including creatininase enzymatic conversion of creatinine into the product creatine which itself is converted to sarcosine by creatine amidinohydrolase (creatinase), followed by oxidation of sarcosine by sarcosine oxidase (SOD) producing hydrogen peroxide. In the presence of peroxidase (POD) the hydrogen peroxide is quantified at 550 nm by the formation of a colored dye. [4]

$$\text{Creatinine} + H_2O \xrightarrow{\text{Creatininase}} \text{Creatine}$$

$$\text{Creatine} + H_2O \xrightarrow{\text{Creatinase}} \text{Sarcosine} + \text{Urea}$$

$$\text{Sarcosine} + H_2O + O_2 \xrightarrow{\text{SOD}} \text{formaldehyde} + \text{Glycine} + H_2O_2$$

$$2H_2O_2 + \text{4-AA} + \text{Toos} \xrightarrow{\text{POD}} 4H_2O + \text{Quinone dye } (\lambda \text{ max } 556)$$

Any endogenous creatine present in the sample is removed by creatinase and sarcosine oxidase during preincubation.

Materials Provided

The reagents necessary for the determination of creatinine (Reagent R1, Reagent R2 and calibrator) are provided.

Materials required but not provided.

Further application sheets for use of the assay on automated clinical chemistry analyzers are available upon request.

Controls for validating the performance of the creatinine reagents and saline for diluting urine samples are not provided.

Reagent Composition

Kit Size	Active Ingredients	Concentration
Reagent R1 (liquid) 1 x 60 mL	Creatinase TOOS SOD	12 IU – 60 IU/mL 0.07 mg – 0.21 mg/mL 4 – 17 IU/mL
Reagent R2 (liquid) 1 x 20 mL	Creatininase 4 – AA POD	135 IU ~ 670 IU/mL 0.3 – 0.9 mg/mL
Creatinine Calibrator 1 x 1 mL	5 mg/dL (442 µM/L) creatinine in 0.9% saline	

Reagent Preparation

The assay reagents (R1, R2, and calibrator) are ready to use, liquid reagents.

Reagent Stability and Storage

Unopened reagents are stable until the expiration date printed on the outer box when stored at 2-8º C. Reagent on-board stability is at least 30 days.

Specimen Collection and Handling

Use fresh patient serum samples. Urine samples should be 10–fold diluted with saline.

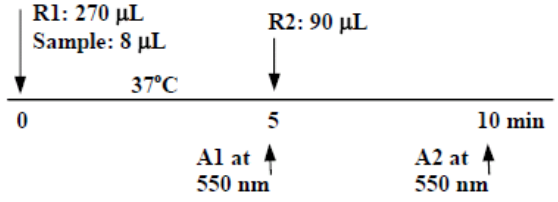
Warnings

1. For *in vitro* diagnostic use only.
2. Specimens and reagents containing human sourced materials should be handles as if potentially infectious, using safe laboratory procedures such as those outlined in Biosafety in Microbiological and Biomedical Laboratories (HHS Publication Number [CDC] 93-8395).
3. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
4. Avoid ingestion and contact with skin or mucous membranes.
5. R1 and R2 reagents contain <0.1% sodium azide, (NaN_3), as a preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azide. On disposal, flush with a large volume pf water to prevent azide build up.
6. Reagents are light sensitive. Do not let bottles remain open. Keep containers tightly stoppered.
7. Do not use the reagents after the expiration date labeled on the outer box.

Assay Procedure

Please contact BQ Kits for instrument – specific parameters.

Test Scheme for Chemistry Analyzers



Calibration
A creatinine calibrator is included with the reagents and, along with 0.9% saline as a zero reference, should be used as directed to calibrate the procedure. The calibrator is traceable to NIST SRM 914a, and should be stored at 2-8º C.

Calibration should be performed at least every 14 days and with each new lot of reagents. Calibration is performed by entering the values as shown on the Instrument Parameter settings provided with the assay kit.

Quality Control
We recommend that each laboratory use creatinine controls to validate the performance of creatinine reagents. A creatinine control is available from BQ Kits. If the results from the control fall outside the acceptable limits, as determined by the assigned value, the tests should not be performed. We recommend that your quality control testing follows federal, state, and local guidelines.

Results [5]
Creatinine concentration is expressed as mg/dL or µmol/L
mg/dL x 88.4 = µmol/L
µmol/L x 0.0113 = mg/dL

When the calibrator values are input as per instructions in mg/dL the results (in mg/dL) are printed out automatically by Hitachi 917. For other instruments, refer to the operator manual for printout instructions.

Muscular young or middle aged adults may have more creatinine in their blood than the norm for the general population. Elderly person, on the other hand, may have less creatinine in their blood than the norm. Infants have normal levels of about 0.2 mg/dL (17.7 µmol/L) or more, depending on their muscle development. A person with only one kidney may have a normal level of about 1.8 or 1.9 mg/dL (159.1 or 168.0 µmol/L). Creatinine levels that reach 2.0 md./dL (176.8 µmol/L) or more in babies and 10.0 mg/dL 9884.0 µmol/L) or more in adults may indicate the need for a dialysis machine to remove wastes from the blood.
Certain drugs can sometimes cause abnormally elevated creatinine levels. It is strongly recommended that each laboratory establish an expected range characteristic for the local population.

Reference Range [6]

	Men	Women
Serum	59-104 µmol/L	45 – 84 µmol/L
	0.67 – 1.17 mg/dL	0.51 - 0.95 mg/dL
Urine*	3540 – 24600 µmol/L	2550 – 20000 µmol/L
	40 - 278 mg/dL	29 - 226 mg/dL

*First Morning Urine

Limitations
A sample with a creatinine level exceeding the linearity limit should be diluted with 0.9% saline and reassayed incorporation the dilution factor in the calculation of the value.

Performance Characteristics
All performance characteristics were determined at our laboratories using Hitachi 917.

Limit of Detection
The limit of detection is 12 µmole/L (0.14 mg/dL).

Linearity
The linearity of the procedure is from 0.14 – 13.56 mg/dL (12-1200 µmole/L) in serum and 0.14 – 141.25 mg/dL (12- 12500 µmole/L) in urine. Results below 0.14 mg/dL (12 µmole/L) are invalid. Results that exceed 141.25 mg/dL (12500 µmole/L) should be diluted 10-fold with saline and retested.

Precision Studies
The assay precision was evaluated according to Clinical Laboratory Standards Institute (formerly NCCLS) EP5-A guideline. In the study, four serum specimens were tested on Hitachi 917 twice daily, in duplicates over 20 days.

Serum Testing	**Within-Run Precision**			
	0.75 mg/dL (66.3 µM)	1.41 mg/dL (125 µM)	4.11 mg/dL (346 µM)	10.28 mg/dL (908.7 µM)
No. of Data Points	80	80	80	80
Mean mg/dL (µM)	0.74 (65.4)	1.38 (122.3)	4.04 (357.5)	10.28 (908.7)
SD mg/dL (µM)	0.015 (1.3)	0.015 (1.37)	0.029 (2.54)	0.015 (1.3)
C_v%	2.1	1.1	0.7	0.1
Serum Testing	**Total Precision**			
	0.75 mg/dL (66.3 µM)	1.41 mg/dL (125 µM)	4.11 mg/dL (346 µM)	10.28 mg/dL (908.7 µM)
No. of Data Points	80	80	80	80
Mean mg/dL (µM)	0.74 (65.4)	1.38 (122.3)	4.04 (357.5)	10.28 (908.7)
SD mg/dL (µM)	0.022 (1.9)	0.026 (2.29)	0.058 (5.11)	0.14 (12.4)
C_v%	3.0	1.9	1.4	1.4

The Assay precision was also evaluated with urine samples with a modified EP10 protocol. For within-run precision, 21 replicates of commercial urine controls were tested. For total precision, 2 runs of each commercial urine control were performed consecutively for 5 days. The samples were diluted ten-fold with 0.9% saline and tested for Creatinine values. The values were multiplied by the dilution factor (i.e. 10) to obtain the final results indicated below.

Urine Testing	**Within-Run Precision**		
	Level 1	Level 2	Level 3
No. of Data Points	21	21	21
Mean mg/dL (µM)	29.09 (2572)	87.1 (7711)	196.7 (17407)
SD mg/dL (µM)	0.1 (8.84)	0.27 (23.60)	0.90 (79.71)
C_v%	0.36	0.31	0.46
Urine Testing	**Total Precision**		
	Level 1	Level 2	Level 3
No. of Data Points	20	20	20
Mean mg/dL (µM)	29.86 (2640)	87.7 (7765)	195 (17265)
SD mg/dL (µM)	0.79 (69.8)	0.67 (59.2)	1.19 (105.2)
C_v%	2.64	0.76	0.60

Assay Accuracy
The performance of this assay was compared with the performance of a legally marketed creatinine assay using serum samples ranging from 0.2 – 13.51 mg/dL (17.7 – 1194.3 µmol/L) and urine samples ranging from 0.14 – 141 mg/dL (12.4 – 12434.4 µmol/L). The serum correlation study was performed with 42 unaltered and 9 altered urine samples. The correlation analyses are presented below for both serum and urine sample matrices.

Accuracy/Serum Samples:
Correlation Coefficient: 0.9981
Slope/Intercept: y = 0.9467x + 0.0643

Accuracy/Urine Samples:
Correlation Coefficient: 0.9968
Slope/Intercept: y = 10002x – 0.0518

Interfering Substances
Interference for the assay was evaluated on Hitachi 917. The following substances normally present in serum produced less than 10% deviation at the listed concentrations: Triglyceride at 1000 mg/dL, Ascorbic acid at 10 mM, Bilirubin at 40 mg/dl, Bilirubin Conjugate at 30 mg/dL, Hemoglobin at 500 mg/dL. The following substances normally present in urine produced less than 10% deviation at the listed concentrations: Triglyceride at 1000 mg/dL, Ascorbic Acid at 10 mM, Bilirubin at 40 mg/dl, Bilirubin Conjugate at 40 mg/dL, Hemoglobin at 1000 mg/dL.

References

1. Tietz, N.W. (Ed): Fundamentals of Clinical Chemistry, W.B. Saunders Co., Philadelphia, 865 (1982)
2. National Kidney Foundation K/DOQI. Clinical Practice Guidelines for chronic kidney disease: evaluation, classification, and stratification. Am J Kidney Dis 2002; 39; S1-S200
3. Badiou S, Dupuy AM, Descomps B, Cirstolead, JP. Comparison between the enzymatic vitros assay for creatinine determination and three other methods adapted on the Olympus analyzer, Journal of Clinical Laboratory Analysis 2003: 17, 235-240
4. Hayes AW. Principles and Methods of Toxicology, Taylor & Francis, 1028 (2001)
5. Cristenson RH, Johnson LJ, Gregory, LC. Appleton and Lange's Outline Review Clinical Chemistry, McGraw-hill Professional, 118 (2001)
6. Mazzachi BC, Peake MJ, Ehrhardt V. Reference Range and Method Comparison Studies for Enzymatic and Jaffe Creatinine Assays in Plasma and Serum and Early Morning Urine. Clin Lab 2000; 46;53-55

Hitachi 917 Parameters
Temperature 37º C

Use the following parameters with saline and calibrator for calibration.

Test	CRE
Assay Code	2 Point End
Assay Point	(10)(16)(34) **
Wavelength (Sub/Main)	(700) (546)
Calibration Type	Linearity
Sample volume (normal)	(8) (0) (0)
Sample volume (Dec.)	(8) (0) (0)
Sample volume (Inc.)	(8) (0) (0)
Diluent	(water) (0)
Reagent vol. R1	(270) (0) (10008) (0)
Reagent vol. R2	(0) (0) (1000) (0)
Reagent vol. R3	(90) (10008) (0) (0)
Reagent vol. R4	(0) (0) (1000) (0)
ABS. Limit	(32000) (Increase)
STD (1) CONC. – Position	(0) – (1)
STD (1) CONC. - Position	(*) – (2)
Expected value (normal value)	
Tech. Limit	0-13260

* Entered by Operator
** Each reading cycle is 18 seconds

Hitachi 717 Parameters
Temperature 37º C

Use the following parameters with saline and calibrator for calibration.

Test	Creatinine
Assay Code	2 Point
Assay Point	(24)-(49) **
Wavelength	700/546
Calibration Method	Linear
Unit	µmol/L
Sample volume	(8)(8)
Reagent vol. R1	(270)(100)(NO)
Reagent vol. R2	(90)(100)(NO)
STD (1) CONC.-POS	(0)-(1)
STD (2) CONC.-POS	(*)-(2)
ABS.Limit	32000-Increase
Expected value (normal Value)	4-20
Tech Limit	0-13260

* Entered by Operator
** Each cycle is 12 seconds

** The above reagent parameter **has not been fully validated** for these analyzers. The parameters are based on BQ Kits' knowledge of the analyzer and reagents, and should perform adequately. However, you should use these parameters as guidelines in conjunction with your Quality Control Program for validation.

Beckman Synchron CX-7 Parameters

Temperature 37º C

Use the following parameters with saline and calibrator for calibration.

CHEMISTRY NAME: CRE
TEST NAM: [CO2] CALCULATION FACTOR: 0

REACTION TYPE: End Point 2 MATH MODEL: Linear
REACTION DIRECTION: Increasing CAL TIME LIMIT: Hrs
UNITS: µmol/L
DECIMAL PRECISION: X.X

NO. OF CALIBRATORS: 2 #1:0
#2: USER DEFINED

PRIMARY WAVELENGTH: 560 NM
SECONDARY WAVELENGTH: 700 NM

SAMPLE VOLUME: 7
PRIMARY INJECT RGT:
A: 230
C: 75
SECONDARY INJECT RGT:
B: None
ADD TIME: 0 SEC

MULTIPOINT SPAN: 1-2: 0.0000

REAGENT BLANK
START READ: 435 SEC; END READ: 451 SEC
LOW ABS LIMIT: -1.5; HIG H ABS LIMIT: 1.5

REACTION
START READ: 480 SEC; END READ: 496 SEC
LOW ABS LIMIT: -1.5; HIGH ABS LIMIT: 1.5

USABLE RANGE
LOWER LIMIT: 0.0
UPPER LIMIT: 13260

SUBSTRATE DEPLETION
INITIAL RATE: -99.99
DELTA ABS: 1.5

Olympus AU400/600/640/2700/5400 Parameters

Temperature 37º C

Use the following parameters with calibrator for calibration.

General
Test Name: CRE Type: Serum Operation: Yes

Sample Volume 8.0 µL Dilution 0 µL Pr-Dilution Rate 1

Reagents: Min OD Max OD

R1 volume 270 µL Dilution 0 µL L: -2.000 H: 2.500
R2 volume 90 µL Dilution 0 µL

Wavelenthg: Pri. 540 Sec. 700 Reagent OD Limit:

Method: END First L: -2.000; First H: 2.500

Reaction Slope: + Last L: -2.000; Last H: 2.500

Measuring Point 1: First 9; Last 27 Dynamic Range:

Measuring Point 2: First; Last L: 0.0 H: 50.0

Linearity 20% Correlation Factor:

No-Lag-Time: No A:1.0000 B:0.0000
Onboard stability Period: 999

Calibration Type AB Formula: Y=AX+B
Counts 2 Process CONC

Cal No.	OD	CONC	Factor/OD-L	Factor/OD-H
Point 1		*		
Point 2				

Advanced Calibration: No
MB Type Factor: Calibration Stability Period: 999

* Entered by Operator