LIQUID LINE

Creatinina

enzimática AA

Enzymatic method for creatinine determination in serum, plasma or urine

SUMMARY

Creatinine, a highly diffusible compound, is eliminated from the body almost exclusively by renal filtration.
Creatinine measurement in serum as well as the endogenous creatinine clearance are important parameters for the diagnosis of various renal disorders.

PRINCIPLE

Based on the following reaction system:

$$\text{creatinine} + H_2O \xrightarrow{\text{creatininase}} \text{creatine}$$

$$\text{creatine} + H_2O \xrightarrow{\text{creatinase}} \text{sarcosine} + \text{urea}$$

$$\text{sarcosine} + H_2O + O_2 \xrightarrow{\text{sarcosine oxidase}} \text{glycine} + HCHO + H_2O_2$$

$$H_2O_2 + \text{4-AP} + \text{TOOS} \xrightarrow{\text{peroxidase}} \text{quinoneimine dye}$$

The intensity of the quinoneimine dye color formed is directly proportional to the creatinine concentration in the sample.

PROVIDED REAGENTS

A. Reagent A: solution containing 36 kU/l creatinase, 11 kU/l sarcosine oxidase, 300 kU/l catalase, 3 kU/l ascorbate oxidase and 20 mmol/l Goods buffer pH 8.2 with 1 mmol/l N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (TOOS).
B. Reagent B: solution containing 4 mmol/l 4-aminophenazone (4-AP), 370 kU/l creatininase, 15 kU/l peroxidase, 0.8 g/l sodium azide and 20 mmol/l Goods buffer pH 8.0.
S. Standard*: 20 mg/l creatinine solution.

NON-PROVIDED REAGENTS

- Wiener lab.'s **Calibrador A plus**.
- Demineralized water.

INSTRUCTIONS FOR USE

Provided Reagents: ready to use.

WARNINGS

Reagents are for "in vitro" diagnostic use.
Use the reagents according to the working procedures for clinical laboratories.
The reagents and samples should be discarded according to the local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: stable in refrigerator (2-10ºC) until the expiration date stated on the box. Do not expose at elevated temperatures for extended periods of time.

SAMPLE

Serum, plasma or urine
a) Collection: obtain serum or plasma in the usual way.
Two-hour or 24-hour urine could also be employed. Use a thoroughly cleaned container, which should be refrigerated (2-10ºC) during collection. Measure diuresis, take an aliquot and perform a 1:50 dilution. For a 2-hour diuresis, multiply measured volume by 12 to calculate the amount of creatinine eliminated during 24 hours.
b) Additives: in case the sample to be used is plasma, it is recommended the use of heparin as anticoagulant.
c) Known interfering substances: no interference has been observed with ascorbic acid up to 100 mg/l, hemoglobin up to 400 mg/dl (4 g/l), bilirubin up to 28 mg/dl (280 mg/l) and triglycerides up to 1250 mg/dl (12.5 g/l).
See Young, D.S. under References for effect of drugs on the present method.
d) Stability and storage instructions: serum or plasma should be separated from cells within 2 hours after collection. If specimens are stored, protected from light, at 2-10ºC, stability is sustained for 3 days.
Urine sample should be stored at 2-10ºC for up 4 days without any preservatives.

REQUIRED MATERIAL (non-provided)

- Spectrophotometer.
- Micropipettes or pipettes for measuring the stated volumes.
- Spectrophotometric cuvettes.
- Water bath at 37ºC.
- Stopwatch.

ASSAY CONDITIONS

- Wavelength: 546 nm
- Reaction temperature: 37ºC

PROCEDURE

Before sample addition, set the instrument to zero O.D. with distilled water. In three spectrophotometric labeled cuvettes B (Blank), S (Standard) and U (Unknown), place as follows:

	B	S	U
Sample	-	-	0.07 ml
Standard	-	0.07 ml	-
Demineralized water	0.07 ml	-	-

** Non-provided with all kit sizes*

Reagent A	2.5 ml	2.5 ml	2.5 ml
Mix and incubate for 5 minutes at 37°C. Measure absorbance at 546 nm (B_1, S_1, and U_1).			
Reagent B	1.25 ml	1.25 ml	1.25 ml
Mix and incubate for 5 minutes at 37°C. Measure absorbance at 546 nm (B_2, S_2, and U_2).			

CALCULATIONS

1) Creatinine in serum (mg/l) = $[(U_2-B_2) - (U_1-B_1) \times k] \times f$

$$f = \frac{20 \text{ mg/l}}{(S_2-B_2) - (S_1-B_1) \times k}$$

where:

$$k = \frac{\text{Blank volume (ml)}}{\text{final volume (ml)}} = \frac{2.57 \text{ ml}}{3.82 \text{ ml}} = 0.673$$

Meaning the compensation by blank dilution by Reagent B addition.

Example:

B_1: 0.123 S_1: 0.145 U_1: 0.164
B_2: 0.137 S_2: 0.365 U_2: 0.412

Standard: 20 mg/l

k = 0.673

$$f = \frac{20 \text{ (mg/l)}}{(0.365-0.137) - (0.145-0.123) \times 0.673} = \frac{20}{0.228-0.022 \times 0.673} =$$

$$= \frac{20}{0.228 - 0.015} = \frac{20}{0.213} = 93.9$$

Creatinine (mg/l) = [(0.412-0.137) - (0.164-0.123) x 0.673] x 93.9 =

= (0.275 - 0.041 x 0.673) x 93.9 = (0.275 - 0.028) x 93.9 =

= 0.247 x 93.9 = 23.19

2) Creatinine in urine (g/24 hrs) =

$$= \frac{\text{Creatinine (mg/l)} \times 50 \times U}{1000} = \frac{\text{Creatinine (mg/l)} \times U}{20}$$

where:
U = diuresis volume expressed in liters/24 hrs
50 = dilution factor
1000 = mg conversion to grams

3) Endogenous Creatinine Clearance (E.C.C.):

$$\text{E.C.C. ml/min} = \frac{\text{Creatinine in urine (g/24 hrs)}}{\text{Creatinine in serum (mg/l)}} \times 694 \text{ ml/min}$$

where:

$$694 \text{ ml/min} = \frac{\text{g/24 hrs}}{\text{mg/l}} = \frac{1{,}000 \text{ mg} \times 1{,}000 \text{ ml}}{1 \text{ mg} \times 1{,}440 \text{ min}} = \frac{1{,}000{,}000 \text{ ml}}{1{,}440 \text{ min}}$$

QUALITY CONTROL METHOD

Each time the test is running, analyze two levels of a quality control material (**Standatrol S-E 2 niveles**) with known creatinine concentration.

REFERENCE VALUES

Creatinine expected values are the following:

Serum or plasma
Men: 7 - 13 mg/l
Women: 6 - 11 mg/l

Urine
Men: 0.8 - 2.0 g/24 hrs
Women: 0.6 - 1.8 g/24 hrs

Endogenous Creatinine Clearance
Men: 94 - 140 ml/min (125 ml/min average)
Women: 72 - 110 ml/min

It is recommended that each laboratory establishes its own reference values.

SI SYSTEM UNITS CONVERSION

Creatinine (mg/l) x 8.84 = Creatinine (umol/l)

PROCEDURE LIMITATIONS

See Known Interfering Substances under SAMPLE.

PERFORMANCE

a) Reproducibility: following the guidelines contained in CLSI EP5-A document the following results were obtained:

Intra-assay precision (n = 20)

Level	S.D.	C.V.
0.98 mg/dl	± 0.014 mg/dl	1.45 %
3.80 mg/dl	± 0.038 mg/dl	0.99 %

Total precision (n = 20)

Level	S.D.	C.V.
0.98 mg/dl	± 0.019 mg/dl	1.99 %
3.80 mg/dl	± 0.044 mg/dl	1.15 %

b) Linearity: reaction is linear up to 170 mg/l (17 mg/dl) of creatinine. For higher values, dilute the sample 1:2 or 1:4 with distilled water and repeat the test. Correct calculations multiplying by the dilution factor used.
c) Analytical sensitivity: in spectrophotometer at 546 nm with 1 cm optical length square cuvettes, for a ΔA minimum of 0.001, the minimum detectable concentration change will be of 1.2 mg/l.

PARAMETERS FOR AUTOANALYZERS

For programming instructions check the user's manual of the autoanalyzer in use.
For calibration, it must be used Wiener lab.'s **Calibrador A plus**.

WIENER LAB. PROVIDES

60 ml: 1 x 40 ml Reagent A
1 x 20 ml Reagent B
(Cat. 1260362)

90 ml: 3 x 20 ml Reagent A
3 x 10 ml Reagent B
(Cat. 1009612)

REFERENCES

- Fossatti, P.; Prencipe, L.; Berti, G. - Clin. Chem. 29:1494 (1983).
- Curtis, C.A.; Ashwood, E.R. - Tietz Fundamentals of Clin. Chem. 5th Ed.: 422, 2001.
- Burtis, C.A.; Ashwood, E.R. - Tietz Textbook of Clin. Chem. 3rd Ed.:1808, 1996.
- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 5th ed., 2000.
- CLSI: Clinical and Laboratory Standards Institute (ex-NCCLS) - Protocols EP 15A, 2001 / EP 17A, 2004.

SYMBOLS

The following symbols are used in packaging for Wiener lab. diagnostic reagents kits.

CE	This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices
EC REP	Authorized representative in the European Community
IVD	"In vitro" diagnostic medical device
	Contains sufficient for <n> tests
	Use by
	Temperature limitation (store at)
	Do not freeze
	Biological risks
⟶	Volume after reconstitution
Cont.	Contents
LOT	Batch code
	Manufactured by:
Xn	Harmful
	Corrosive / Caustic
Xi	Irritant
	Consult instructions for use
Calibr.	Calibrator
CONTROL	Control
CONTROL +	Positive Control
CONTROL −	Negative Control
REF	Catalog number

Wiener Laboratorios S.A.I.C.
Riobamba 2944
2000 - Rosario - Argentina
http://www.wiener-lab.com.ar
Dir. Téc.: Viviana E. Cétola
Bioquímica

A.N.M.A.T. Registered product
Cert. Nº: 5708/05

Wiener lab.

2000 Rosario - Argentina

000/03UR0130509