



Creatinina

cinética AA

Kinetic method for the determination of creatinine in serum, plasma or urine

SUMMARY

Creatinine, a highly diffusible compound, is eliminated from the body almost exclusively by renal filtration.

Creatinine detection in serum as well as the endogenous creatinine clearance are important parameters for the diagnosis of various renal disorders.

PRINCIPLE

Creatinine reacts with the alkaline picrate (Jaffe reaction) yielding a red chromogen. Being this a first order kinetic reaction, its velocity under controlled conditions, is directly related to creatinine concentration in the sample. On the other hand, it has been proved that non-creatinine chromogens, which interfere with most of the conventional techniques, react within 30 seconds from the beginning of the reaction. Thus, increasing intensity of color reaction 30 seconds and 5 minutes is due exclusively to creatinine.

PROVIDED REAGENTS

A. Reagent A: 12.7 mmol/l picric acid and 8.4 mmol/l sodium lauryl sulphate solution.

B. Reagent B: 53 mmol/l borate and 970 mmol/l sodium hydroxide solution.

S. Standard*: 20 mg/l creatinine solution.

NON-PROVIDED REAGENTS

Wiener lab.'s **Calibrador A plus**.

INSTRUCTIONS FOR USE

Reagent A: ready to use. At low temperature, the reagent may precipitate or form sediment. In that case, place in a water bath at 37°C for a few of minutes before using.

Reagent B: ready to use.

Working Reagent: according to the working volume, mix four parts Reagent A and one part Reagent B. Label and date.

At low temperature, the Working Reagent may precipitate or form sediment. In that case, place in a water bath at 37°C for a few of minutes before using.

WARNINGS

Reagents are for "in vitro" diagnostic use.

Reagent B: corrosive. H315+H320: Causes skin and eye irritation. H314 Causes severe skin burns and eye damage. P262 Do not get in eyes, on skin, or on clothing. P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P302 + P352 IF ON SKIN: Wash with plenty of soap and water. P280 Wear protective gloves/protective

clothing/eye protection/face protection.

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 at room temperature (< 25°C) until expiration date stated on the box.

Working Reagent: premixed and in plastic bottle, is stable for one week at room temperature. For autoanalyzers that use a single Working Reagent (premixed), refer to the specific applications for each instrument.

Avoid prolonged exposure to air, keeping the Working Reagent tightly capped when unused.

SAMPLE

Serum, plasma or urine

a) Collection: obtain serum or plasma in the usual way. Two hour or 24-hour urine could be employed. Use a thoroughly cleaned container, which should be store at 2-10°C during collection. Measure diuresis, take an aliquot and perform a 1:50 dilution in distilled water. For a 2-hour diuresis, multiply measured volume by 12 to calculate the amount of creatinine eliminated during 24 hours.

b) Additives: heparin or EDTA.

c) Known interfering substances: no interference has been observed in serum or plasma with hemoglobin up to 0.78 g/dl (7.8 g/l), triglycerides up to 1700 mg/dl (17 g/l) and bilirubin up to 24 mg/dl (240 mg/l). In urine sample, no interferences have been observed with proteins up to 500 mg/dl (5 g/l), ascorbic acid up to 100 mg/dl (1 g/l) nor ketones up to 4 mmol/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 and stored at 2-10°C for up to 72 hours. Urine sample could be stored at 2-10°C for up to 4 days without preservatives.

REQUIRED MATERIAL (non-provided)

- Spectrophotometer capable of reading at 500 ± 10 nm.
- Micropipettes or pipettes for measuring the stated volumes.
- Spectrophotometric cuvettes.
- Water bath at 25°C.
- Stopwatch.

ASSAY CONDITIONS

- Wavelength: 500 nm
- Reaction temperature: 25°C

Temperature control is not critical, oscillating between 22 and 28°C. See PROCEDURE LIMITATIONS.

- Sample volume: 0.2 ml
- Working Reagent volume: 1.2 ml
- Final reaction volume: 1.4 ml

PROCEDURE

I- TECHNIQUE FOR SERUM OR PLASMA

Bring the Working Reagent at room temperature (25°C). Set the instrument to zero O.D. with distilled water. In two spectrophotometric labelled cuvettes, place S (Standard) and U (Unknown), as follows:

	S	U
Working Reagent	1.2 ml	1.2 ml
Standard	0.2 ml	-
Sample	-	0.2 ml

Mix at once starting stopwatch and incubate for 5 minutes. Measure absorbance (S_1 and U_1) after exactly 30 seconds at 500 ± 10 nm and measure absorbance again (S_2 and U_2) after 5 minutes (4 minutes 30 seconds after first reading).

II- TECHNIQUE FOR URINE

Measure diuresis and take an aliquot. Performed a 1:50 dilution in deionized water. Then follow the technique I.

CALCULATIONS

1) Creatinine in serum (mg/l) = $(U_2 - U_1) \times f$

$$f = \frac{20 \text{ mg/l}}{S_2 - S_1}$$

2) Creatinine in urine (g/24 hrs) = $\frac{U_2 - U_1}{S_2 - S_1} \times V$

where:

V = diuresis volume in liters/24 hrs

The formula comes from:

$$\text{Creatinine in urine (g/24 hrs)} = \frac{U_2 - U_1}{S_2 - S_1} \times 0.020 \text{ g/l} \times 50 \times V$$

where:

0.020 g/l = creatinine concentration in Standard

50 = dilution factor

To express creatinine in urine in "mg/kg/24 hrs":

$$\frac{\text{Creatinine in urine (g/24 hrs)} \times 1000 \text{ mg/g}}{\text{Patient weight (kg)}}$$

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

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

where:

$$694 \text{ ml/min} = \frac{\text{g/24 hs}}{\text{mg/l}} = \frac{1000 \text{ mg} \times 1000 \text{ ml}}{1 \text{ mg} \times 1440 \text{ min}} = \frac{1,000,000 \text{ ml}}{1440 \text{ min}}$$

To express E.C.C. in "ml/min/1.73 m²":

$$\frac{\text{E.C.C. (ml/min)} \times 1.73}{\text{Patient's body surface (m}^2\text{)}}$$

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

Serum or plasma:

Men: 7 - 13 mg/l

Women: 6 - 11 mg/l

Urine:

Men: 14 - 26 mg/kg/24 hrs

Women: 11 - 20 mg/kg/24 hrs

E.C.C.:

Men: 94 - 140 ml/min/1.73 m²

Women: 72 - 110 ml/min/1.73 m²

It is recommended that each laboratory establish 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.

Other sources of erroneous results are:

- Temperature: reaction temperature admits a variation between 22 and 28°C, however a difference between the incubation temperature of the Standard and the Samples decreases method's precision.
- Reading time: slight variations in time measurement considerably affect the method's accuracy. Readings should be performed exactly 30 seconds after mixing the sample with the reagent and after 5 minutes (4 minutes 30 seconds after first reading).

PERFORMANCE

a) **Reproducibility:** the following was obtained according to the guidelines contained in CLSI (ex NCCLS) EP15-A document:

Intra-assay precision (n = 20)

	Level	S.D.	C.V.
Serum	7.1 mg/l	± 0.27 mg/l	3.8 %
	14.7 mg/l	± 0.32 mg/l	2.2 %
	54.9 mg/l	± 1.09 mg/l	2.0 %
Urine	440 mg/l	± 9.35 mg/l	2.1 %
	2.620 mg/l	± 62.4 mg/l	2.4 %
	5.060 mg/l	± 136 mg/l	2.7 %

Total precision (n = 20)

	Level	S.D.	C.V.
Serum	7.1 mg/l	± 0.30 mg/l	4.3 %
	14.7 mg/l	± 0.48 mg/l	3.3 %
	54.9 mg/l	± 1.47 mg/l	2.7 %
Urine	440 mg/l	± 20.5 mg/l	4.6 %
	2,620 mg/l	± 119 mg/l	4.5 %
	5,060 mg/l	± 177 mg/l	3.5 %

b) Linearity: reaction in linear up to 90 mg/l creatinine. For higher values, dilute sample 1:2 or 1:4 with saline solution and repeat the test. Correct calculations multiplying by the dilution factor used.

c) Detection limit: the minimum detectable activity change is 4,5 mg/l.

PARAMETERS FOR AUTOANALYZERS

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

WIENER LAB. PROVIDES


- 250 ml (2 x 100 ml Reagent A + 2 x 25 ml Reagent B) (Cat. N° 1260360)
- 200 ml (4 x 40 ml Reagent A + 2 x 20 ml Reagent B) (Cat. N° 1009329)
- 200 ml (4 x 40 ml Reagent A + 2 x 20 ml Reagent B) (Cat. N° 1009254)
- 300 ml (4 x 60 ml Reagent A + 3 x 20 ml Reagent B) (Cat. N° 1009611)

REFERENCES

- Owen, J.A.; et al. - Biochem. J. 58:426 (1954).
- Henry, R.J. et al. - Clinical Chemistry, Principles and Techniques, 2nd ed., Harper and Row Publishers Inc., N.Y. (1974).
- Butler, A.R. - J. Chem. Soc. (Perkin Trans. II), 853 (1975).
- Labbé, D et al. - Ann. Biol. Clin. 54:285 (1996).
- Burtis, CA; Ashwood, ER - Tietz Fundamentals of Clin. Chem., 5th ed., 2001.
- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 5th ed., 2000.
- CLSI: Clinical and Laboratory Standards Institute (ex-NCCLS) - Protocol EP15-A, 2001 / EP 17A, 2004.


SYMBOLS

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

 This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices

 Authorized representative in the European Community

 "In vitro" diagnostic medical device


 Contains sufficient for <n> tests

 Use by


 Temperature limitation (store at)

 Do not freeze

 Biological risks

 Volume after reconstitution

 Contents

 Batch code

 Manufactured by:

 Harmful

 Corrosive / Caustic

 Irritant

 Consult instructions for use


 Calibrator

 Control

 Positive Control

 Negative Control

 Catalog number

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