CREATININE (Enzymatic Method)

Introduction

Creatinine measurement is used in the diagnosis and follow-up of acute and chronic renal disease, measurement of glomerular filteration rate (GFR),or evaluation of the status of renal dialysis patients. Urine creatinine analysis; it is used to calculate creatinine clearance, confirm completion of 24-hour aggreagation, or provide a reference quantity for other analytes, such as calculating the albumin/creatinine ratio.

Method Principle

The creatinine present in the sample is hydrolyzed to creatinine by creatinase. Next, creatinine is hydrolyzed by creatinase to sarcosine and urea. Sarcosine formed as result of this reaction is oxidized by sarcosine oxidase to glycine and formaldehyde, and in the meantime, hydrogen peroxide is released. 2 Hydrogen peroxide reacts with 4-aminoantipyrine and ESPMT in the presence of peroxide to form a quinoneimine dye.

Enzymatic method employs a two-reagent system which eliminates interference by endogenous creatinine and ascorbic acid.

$$\text{Creatinine} + H_2O \xrightarrow{\text{Creatinine Amidohydrolase}} \text{Creatinine}$$

$$\text{Creatinine} + H_2O \xrightarrow{\text{Creatinine Amidohydrolase}} \text{Sacrosine} + \text{Urea}$$

$$\text{Sacrosine} + H_2O_2 + O_2 \xrightarrow{\text{Sarcosine Oxidase}} \text{Glycine} + HCHO + H_2O_2$$

$$2\,H_2O_2 + \text{4-AAP} + \text{ESPMT} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_2O$$

Kit Contents

R1 – Creatinine Reagent
R2 – Creatinine Reagent
Calibrator (Conc. As on Vial)

Specimen

Serum, Plasma or Urine

Serum/plasma creatinine is stable for 3 days at 15-25°C, 7 days at 2-8°C and 3 months at -20°C.

Urine creatinine is stable for 2 days at 15-25°C, 6 days at 2-8°C and 6 months at -20°C.

Manual Procedure

Wavelength 546 nm
Method Fixed Kinetic/Increasing/Two Point

Read against reagent blank

	Blank	Calibrator	Sample
Reagent R1	450 µl	450 µl	450 µl
Calibrator		10 µl	
Sample			10 µl

Mix and incubate for 5 min at 37°C. Then add:

Reagent R2	150 µl	150 µl	150 µl

Mix and after 1 minute of incubation measure the change of absorbance per minute (ΔA/min.) during next 3 minutes.

Assay Guidelines for Analyzers

Mode	Two Point
Wavelength (Filter	546 nm
Secondary Wavelength/Biochromatic	None
Reagent Blank	No
Sample Blank	No
Sample Vol.	10 µl
Reagent Vol.	600 µl (R1–450 µl R2-150 µl)
Delay Time	60 sec
Measuring Time/ Interval	180 sec
Reagent Blank Abs. (Max)	<0.300 abs
Calibration Method	1-point/Factor
Factor	Calculated by system
Calibrator (Conc)	See Vial Label
Linearity	150 mg/dL
Temp.	37°C
Unit	mg/dL
Ref. Low (Serum) Male/Female	0.70/0.55 mg/dl
Ref. High (Serum) Male/Female	1.10/1.00 mg/dl

Calculation

Creatinine concentration (mg/dL) $= \frac{\Delta A(A2-A1)Sample}{\Delta A(A2-A1)Calibrator} Xn$

Where n= Calibrator concentration

Take dilution factor into account for calculation of creatinine concentration in urine.

Conversion factor: mg/dLx88.40 = µmol/L
Mg/dLx0.0884 = µmol/L

Linearity: up to 150 mg/dL, if creatinine concentration exceeds 150 mg/dL, dilute the sample with 0.9% saline and repeat the assay. Multiply the result by the dilution factor.

Quality Control

To ensure adequate quality control, each run should include assayed normal and abnormal controls. The assay requires the use of a Creatinine Enzymatic Standard Calibrator STIf commercial controls are not available it is recommended that known value samples be aliquoted, frozen and used as controls.

ReferenceValues

	Men	Women	Unit
Serum, Plasma	0.70-1.10	0.55-1.00	mg/dl
Urine	11-19	5-16	Mg/kg/24hr*

*Equation for the calculation of Creatinine in a 24-hour urine specimen.

mg/dLx10x(V/W)=mg/kg/24hr

mg/dLx88.4x(V/W)=µmol/kg/24hr

KEE DIAGNOSTICS

V: 24-hour urine volume in L
W: patient weight in kg
It is recommended for each laboratory to establish its own reference ranges for local population.

Performance Characteristics

-Limit of Detection (LoD) & Limit of Quantification (LoQ)

	Serum/Plasma	Urine
LoD	0.02 mg/dL	0.05 mg/dL
LoQ	0.08 mg/dL	2.0 mg/dL

- **Precision**

a) Serum/Plasma

		Mean	Within run	
	N	mg/dL	SD	CV%
Low Level	20	0.43	0.01	1.73
Medium Level	20	1.1	0.14	1.31
High Level	20	4.66	0.04	0.85

b) Urine

		Mean		Within run	
	N	mg/dL	µmol/L	SD	CV%
Low Level	20	83	7.3	0.01	1.73
Medium Level	20	159	14.1	0.14	1.31
High Level	20	308	27.2	0.04	0.85

- **Specificity/Interferences:**

a) Serum/Plasma

Studies have been performed to determine the level of interference from different compounds. Recovery is within ±10%.

	Serum/Plasma	Urine
Initial Value	1.2 mg/dL	80 mg/dl & 150 mg/dl
Unconjugated Bilirubin	40 mg/dL	
Conjugated Bilirubin	16 mg/dL	29.5 mg/dL
Haemoglobin	500 mg/dL	500 mg/dL
Triglycerides	3000 mg/dL	
Uric Acid	20 mg/dL	
Glucose	500 mg/dL	5000 mg/dL
Creatinine	50 mg/dL	
Ascorbic Acid	100 mg/dL	20 mg/dL
Methyldopa		10 mg/dL
Calcium dobesilate		50 mg/dL
pH		Between 2.5 & 12

- Results can be falsely lowered by significant levels in sample of NAC (N-Acetyl-Cysteine), NAPQI (metabolite ofacetaminophene (paracetamol)) of metamizole.
- Many other substances and drugs may interfere
- The results of this assay should only be interpreted in conjunction with other diagnostic test results, clinical findings and the patient's medical history.

Waste Management

Please refer to local legal requirements.

Literature

1. Chernecky CC, Berger BJ, eds. (2004).Laboratory Tests and Diagnostic Procedures, 4th ed. Philadelphia: Saunders.
2. Fossati P, Prencipe L, Berti G. Enzymatic creatinine assay: anew colorimetric method based on hydrogen peroxide measurement. ClinChem 1983, 29:1494-1496
3. Mazzachi BC, Peake M, ErhardtV.Reference range and method comparison studies for enzymatic and Jaffé Creatinine assays in plasma and serum and early morning urine. Clin Lab 2000, 46:53-55.
4. Junge W, Wilke B, Halabi A, Klein G.Determination of reference intervals for serum creatinine, creatinine excretion and creatinine clearance with an enzymatic and amodifiedJaffé method. ClinChimActa 2004, 344:137-148

Symbols

IVD In Vitro Diagnostics
Caution
LOT Batch No.
Product Expiry Date
CONT Content
Manufactured By
Read Instructions
Date of Manufacture
Storage Temperature
Keep Dry
REF Catalogue No.
Fragile
Keep away from sun light

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