Audit Diagnostics

CE

CREATININE TRINDER

Creatinine-TR

Enzymatic

Quantitative determination of creatinine

IVD

Store at 2-8ºC

PRINCIPLE OF THE METHOD

In the first reaction, creatinase and sarcosine oxidase were used in the enzymatic hydrolysis of endogenous creatine to produce hydrogen peroxide, that is eliminated by catalase. In the second reaction, the catalase is inhibitred by sodium azide, and creatinase and 4- aminoantipyrine (4-AA) were added, and only the creatine generated from creatinine by creatininase was hydrolyzed sequentially by creatinase and sarcosine oxidase to produce hydrogen peroxide. This newly-formed hydrogen peroxide was measured in a coupled reaction catalyzed by peroxidase, with N-ethyl-n-sulphopropyl-mtoluidine (TOPS)/4-AA as a chromogen.

CLINICAL SIGNIFICANCE

Creatinine is the result of the degradation of the creatine, component of muscles, it can be transformed into ATP, that is a source of high energy for the cells. The creatinine production depends on the modification of the muscular mass, and it varies little and the levels usually are very stable.
Is excreted by the kidneys. With progressive renal insufficiency there is retention in blood of urea, creatinine and uric acid.
Elevate creatinine level may be indicative of renal insufficiency[2].
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

R 1	MOPS 25 mmol/L, TOPS 0,5 mmol/L, Creatinase 10 KU/L, Sarcosine Oxidase 5 KU/L Catalase 3 KU/L, EDTA 1mmol/L, pH 7,5.
R 2	MOPS 90 mmol/L, Creatininase 30 KU/L, peroxidase KU/L, pH 7,5. Azida sódica 0,5 g/L.
CREATININE CAL	Creatinine aqueous primary standard 2 mg/dL.

PRECAUTIONS

CAL: H290-May be corrosive to metals.
Follow the precautionary statements given in MSDS and label of the product.

PREPARATION

R1 and R2 are ready to use.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC, protected from light and contaminations prevented during their use.
R1 and R2 are stable 8 weeks after opening bottle.

ADDITIONAL EQUIPMENT

- Spectrophotometer o photometer measuring at 545±20 nm
- Cell holder thermostable at 37ºC
- General laboratory equipment.

SAMPLES

- Serum or plasma[1].
- Urine (24 h)[1]: Dilute fresh urine 1/50 with disitlled water.
  Multiply the result by 50 (sample dilution factor).
  Creatinine is stable 1 day at 2-8ºC.

PROCEDURE

1. Assay conditions:
   Wavelength: . . . . . 545 nm (525-565)
   Cuvette: . . . . . 1 cm light path
   Temperature: . . . . . 37ºC (±0,1ºC)
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette (Note 3):

	Blank	Standard (Note 1,2)	Sample
R1 (µL)	450	450	450
Sample (µL)	10	10	10

4. Mix and incubate 5 minutes.
5. Read the absorbance (A_1) of the standard and the samples, at 545nm against the blank.
6. Add:

	Blank	Standard	Sample
R2 (µL)	150	150	150

7. Mix and incubate 5 minutes.
8. Read the absorbance (A_2) of the standard and the samples, at 545nm against the blank.

CALCULATIONS

$$\text{Creatinine} = \frac{\Delta A \text{ Sample} \times k - \Delta A \text{Blank} \times k}{\Delta A \text{ Standard} \times k - \Delta A \text{Blank} \times k} \times C$$

K= 0,754= 460 µL/610 µL
C= Concentration of the standard
$\Delta A = A_2 - A_1$

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures: CONTROL Normal and Pathologic .
If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[1]

Serum or plasma:

Men	0,9 - 1,3 mg/dL
Women	0,6 - 1,1 mg/dL
Urine:	
Men	14 - 26 mg/Kg/24 h
Women	11 -20 mg/Kg/24 h

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: From *detection limit* of 0,00 mg/dL to *linearity limit* of 180 mg/dL.
If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.
Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (mg/dL)	0,87	3,82	0,87	3,75
SD	0,01	0,06	0,02	0,06
CV (%)	1,63	1,44	2,31	1,72

Sensitivity: 1 mg/dL = 0,0226 (ΔA)
Accuracy: Results obtained using Audit Diagnostics these reagents did not show systematic differences when compared with other commercial reagents or withHPLC method.
The results obtained using 50 samples were the following:
Correlation coefficient $(r)^2$: 0,9730
Regression equation: y= 1,066x - 0,020.
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

No interferences were observed with haemoglobin until 5 g/dL, bilirubin 40 mg/dL.
Other drugs and substances may interfere[3,4].

NOTES

1. CREATININE CAL: Proceed carefully with this product because due its nature it can get contamined easily.
2. Calibration with the aqueous Standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
3. Use clean disposable pipette tips for its dispensation.
4. **Audit Diagnostics has instruction sheets for several automatic analyzers.**

BIBLIOGRAPHY

1. Fossati et al. Clin Chem 1983;29:1494-1496.
2. Tietz Textbook of Clinical Chemistry, 3rd edition. Burtis CA, Ashwood ER. WB Saunders Co.,1999.
3. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
4. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.

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