Manual Procedure

Automated procedure on request

MEDICHEM MIDDLE EAST
Clinical Chemistry Reagents
Liquid Stable Reagents

Cat. No. 12330 R1 2 x 50 ml
For 100 tests R2 1 x 20 ml

Creatinine PAP

Enzymatic kinetic colorimetric method

Liquid Reagents

Test principle

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

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

$$\text{Sarcosine} + H_2O + O_2 \xrightarrow{\text{Sarcosinoxidase}} \text{Glycine} + HCHO + H_2O_2$$

$$H_2O_2 + \text{TOPS} + \text{4-Aminophenazone} \xrightarrow{\text{Peroxidase}} \text{Quinonimin} + \text{Urea} + H_2O$$

Serum Creatinine is hydrolyzed to creatine in the presence of creatinase enzyme, then creatine is hydrolyzed to sarcosine and urea in the presence of creatinase enzyme, the sarcosine is converted to glycine, formaldehyde and hydrogen peroxide by sarcosine oxidase.
The oxid active condensation of TOPS and 4-aminophenazone in the presence of peroxidase (POD) and hydrogen peroxide produces a rose color dye which is measured at 550 nm. The intensity of the color formed is directly proportional to the Creatinine concentration of the sample.

Concentrations in the test

Reagent R1		
Buffer (pH = 7.65)	25	mmol/L
Creatinase	≥ 14.5	KU/L
Sarcosinoxidase	≥ 11.3	KU/L
Ascorbatoxidase	≥ 3.0	KU/L
TOPS (N-ethyl-n-sulfopropyl-m-toluodine)	0.012	mmol/L
Preservative/Detergent		
Reagent R2		
Buffer (pH = 7.65)	25	mmol/L
Potassium hexacyanoferrat (II)	10	µmol/L
Peroxidase	≥ 2	KU/L
Creatininase	≥ 120	KU/L
Aminophenazone	2	mmol/L
Preservative l/Detergent		

Standard : The concentration as indicated on vial.

Stability of reagents

Reagent R1: *liquid, ready to use.*
Reagent R2: *liquid, ready to use.*
All reagents are stable up to expiry date given on the label when stored at +2 ➔ +8°C.

Note: Don't use if the reagent is turbid.

Specimen collection and handling

Non-hemolyzed serum, EDTA or heparinized plasma.
Stability: Serum: 7 days at 2 - 8°C, Urine 24/ hrs: 6 days at 2 - 8°C.
Urine: Dilute (1+49) with double distilled water, and multiply result by 50.

Calibrator / Standard

MediCal U Cat. No. 15011
Creatinine STD. Cat. No. 16091

Quality control

Meditrol N Cat. No. 15171
Meditrol P Cat. No. 15181

Procedure

Wavelength	Hg 546 (540 - 560 nm)
Spectrophotometer	550 nm
Cuvette	1 cm light path
Temperature	37°C
Measurement	against air or distilled water
Reaction	fixed time

Assay

	Calibrator/Standard	Sample
Calibrator / Standard	50 µl	-
Sample	-	50 µl
Reagent R1	1000 µl	1000 µl
Mix, incubate for 5 min. at 37 °C.		
Reagent R2	200 µl	200 µl
Mix well, incubate for 30 Sec. at 37°C and read the absorbance (A_1), start stop watch and read the absorbance (A_2) exactly after 3 min.		

Calculation

$$\text{Conc.}_{\text{Creatinine}}\ (\text{mg/dl}) = \frac{(A_2 - A_1)_{\text{Sample}}}{(A_2 - A_1)_{\text{Cal./STD}}} \times \text{Conc.}_{\text{Cal./STD}}\ (\text{mg/dl})$$

$$\text{Creatinine in Urine /24 hr.} = \frac{\text{Creatinine mg/dl (Urine) x (vol./ L) }_{\textbf{Urine /24 hr.}}}{100}\ (\text{g/24 hr.})$$

$$\text{Creatinine Clearance} = \frac{\text{Creatinine mg/dl (Urine) x (vol./ml) }_{\textbf{Urine /24 hr.}}}{\text{Creatinine mg/dl (Serum) x 1440}}\ (\text{ml/min.})$$

Linearity

Up to 20 mg/dl.
If the result exceeds 20 mg/dl, repeat the test using diluted sample (1+1) with sodium chloride solution (0.9 %) and multiply the result by 2.

Interferences

1. Conjugated or direct Bilirubin: No significant interference up to 40 mg/dl.
2. Hemolysis: No significant interference of hemoglobin up to 500 mg/dl.
3. A number of drugs and substances affect creatinine accuracy. See Young, *et al*.

Precautions

1. The reagents contain toxic substances. Don't pipette by mouth. Avoid all contact.
2. Avoid any contamination of R1 with R2.

Reference range

Serum

New born		< 1.2	mg/dl
≤ 6 mth.		< 0.9	mg/dl
≥ 7 mth.		< 1.0	mg/dl
Adults	women	< 0.9	mg/dl
	men	< 1.1	mg/dl

Urine

Urine Random	90 - 300	mg/dl
Urine /24 hr.	0.6 - 2.0	g/24 hr.

Creatinine Clearance

Men / Women	71 - 151	ml/min

References

1. Piero Fossati, Lorenzo Principe und Giovanni Berti.Clin.Chem.29/8,1494(1983).
2. Borner, U. und Al., J. Clin. Chem. Clin. Biochem. 17 (1979).
3. Sarre, H., Nierenkrankheiten. Georg Thieme Verlag Stgt. (1959).
4. New water soluble reagent for the enzymatic photometric determination of hydrogen peroxide K. Tamaoku, et al. anal. clin. acta 136, 121 (1982).
5. Young, DS., Effects of Drugs on Clinical Laboratory Tests, fifth edition 2000, AACC Press, Washington, D.C.