CREATININE (CREA)

MANUAL
RX MONZA

INTENDED USE

For the quantitative *in vitro* determination of creatinine in serum, plasma or urine. This product is suitable for manual use and on the Rx Monza analyser.

Cat. No.			
CR 510	CAL.	Standard	1 x 5.5 ml
200 ml	R1a.	Picric Acid	1 x 100 ml
	R1b.	Sodium Hydroxide	1 x 100 ml
GTIN:	05055273201901		
CR 524	CAL.	Standard	1 x 30 ml
6 x 500 ml	R1a.	Picric Acid	3 x 500 ml
	R1b.	Sodium Hydroxide	3 x 500 ml
GTIN:	05055273201918		

CLINICAL SIGNIFICANCE

Creatinine is derived from creatine and creatine phosphate in muscle tissue and may be defined as a nitrogenous waste product. Creatinine is not reutilised but is excreted from the body in the urine via the kidney. It is produced and excreted at a constant rate which is proportional to the body muscle mass. As a consequence of the way in which creatinine is excreted by the kidney, creatinine measurement is used almost exclusively in the assessment of kidney function. Creatinine is regarded as the most useful endogenous marker in the diagnosis and treatment of kidney disease.
Creatinine is measured primarily to assess kidney function and has certain advantages over the measurement of urea. The plasma level of creatinine is relatively independent of protein ingestion, water intake, rate of urine production and exercise. Since its rate of production is constant, elevation of plasma creatinine is indicative of under-excretion, suggesting kidney impairment. Depressed levels of plasma creatinine are rare and not clinically significant.

COLORIMETRIC METHOD[1]

PRINCIPLE

Creatinine in alkaline solution reacts with picric acid to form a coloured complex. The amount of the complex formed is directly proportional to the creatinine concentration.

SAMPLE COLLECTION AND PREPARATION

Serum, Heparinized or EDTA plasma. Stable for 7 days at +2 to + 8°C.
Urine collected without additives. Urine samples should be diluted 1 + 49 with distilled water. Stable for 4 days at +2 to +8°C.

REAGENT COMPOSITION

Contents		Initial Concentrations of Solutions
CAL.	**Standard**	See lot specific insert
R1a.	**Picric Acid**	35 mmol/l
R1b.	**Sodium Hydroxide**	0.32 mol/l

SAFETY PRECAUTIONS AND WARNINGS

For *in vitro* diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

Solution R1a contains picric acid which is poisonous.
Solution R1b contains sodium hydroxide which is caustic.

Solution R1b classified as hazardous according to Regulation (EC) No. 1272/2008 (CLP).

DANGER

Causes severe skin burns and eye damage.

Wear protective gloves/protective clothing/eye protection/face protection.
IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Store locked up.

Safety data sheets available on request.

Please dispose of all Biological and Chemical materials according to local guidelines.

The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.

STABILITY AND PREPARATION OF REAGENTS

CAL. Standard
Supplied ready to use. Stable to expiry date when stored at +2 to +25°C.

R1a. Picric Acid
Supplied ready to use. Stable to expiry date when stored at +15 to +25°C.

R1b. Sodium Hydroxide
Supplied ready to use. Stable to expiry date when stored at +15 to +25°C.

STABILITY AND PREPARATION OF WORKING REAGENT

Mix equal volumes of Solutions R1a + R1b. Stable for 3 days at +15 to +25°C.

MATERIALS PROVIDED

Standard
Picric Acid
Sodium Hydroxide

MATERIALS REQUIRED BUT NOT PROVIDED

Pipetting devices for the delivery of 50 µl, 100 µl, 200 µl, 1 ml and 2 ml.
Timing device and water bath or heating block to maintain temperature at +25, +30 or +37°C.
Spectrophotometer with wavelength capability of 490 to 510 nm.
Randox Assayed Multisera Level 2 (Cat. No. HN 1530) and Level 3 (Cat. No. HE 1532).
Randox Calibration Serum Level 3 (Cat. No. CAL 2351).

PROCEDURE NOTES

Reaction rate and absorbance of the reaction product are very sensitive to temperature. The specified temperature must therefore be maintained.

PROCEDURE

Using fresh ddH_2O perform a new Gain Calibration in cuvette mode. Select CREA in the Run Test screen and carry out a water blank as instructed.

Pipette into a cuvette:

	Reagent Blank S0	Standard S1	Sample
ddH_2O	50 µl	-	-
Standard	-	50 µl	-
Sample	-	-	50 µl
Working Reagent	500 µl	500 µl	500 µl

Mix, insert into the Rx Monza flowcell holder and press Read.

FOR MANUAL USE

Wavelength:	492 (490-510 nm)
Cuvette:	1 cm light path
Temperature:	+25/+30/+37°C
Measurement:	against air

Pipette into cuvette:

	Standard		Sample	
	Macro	Semi Micro	Macro	Semi Micro
Working reagent	2.0 ml	1.0 ml	2.0 ml	1.0 ml
Standard solution	0.2 ml	0.1 ml	-	-
Sample	-	-	0.2 ml	0.1 ml

Mix and after 30 seconds read the absorbance A_1 of the standard and sample. Exactly 2 minutes later, read absorbance A_2 of standard and sample.

CALIBRATION FOR RX MONZA

Recommended on change of reagent lot or as indicated by quality control procedures, using supplied CAL Standard in kit or Randox Calibration Serum Level 3.

MANUAL CALCULATION

$A_2 - A_1 = \Delta A_{sample}$ or $\Delta A_{standard}$

Concentration of creatinine in serum or plasma.

$$\frac{\Delta A_{sample}}{\Delta A_{standard}} \times \text{Standard conc.(µmol/l)} = \text{µmol/l}$$

$$\frac{\Delta A_{sample}}{\Delta A_{standard}} \times \text{Standard conc (mg/dl)} = \text{mg/dl}$$

Concentration of creatinine in urine.

$$\frac{\Delta A_{sample}}{\Delta A_{standard}} \times \text{Standard conc.(µmol/l) x 0.05} = \text{mmol/l}$$

$$\frac{\Delta A_{sample}}{\Delta A_{standard}} \times \text{Standard conc (mg/dl) x 50} = \text{mg/dl}$$

$$\textbf{Creatinine Clearance} = \frac{\text{mg creatinine/dl urine x ml urine 24 hrs}}{\text{mg creatinine/dl serum x 1440}} \text{ [ml/min]}$$

STANDARDISATION

Randox Calibration Serum Level 3 is traceable to creatinine reference materials NIST 909b and NIST 967.

QUALITY CONTROL

Randox Assayed Multisera, Level 2 and Level 3 are recommended for daily quality control. Two levels of controls should be assayed at least once a day. Values obtained should fall within a specified range. If these values fall outside the range and repetition excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check cleanliness of all equipment in use.
3. Check water, contaminants i.e. bacterial growth may contribute to inaccurate results.
4. Check reaction temperature.
5. Check expiry date of kit and contents.
6. Contact Randox Laboratories Technical Services, Northern Ireland +44 (0) 28 94451070.

INTERFERENCE

Haemolysis interferes with the test. Do not use lipaemic sera. The method is subject to interferences from high levels of reducing substances. Boiling urine before testing may help reduce this interference.

NORMAL VALUES[2]

Serum:	Men	53 - 97 µmol/l (0.6 - 1.1 mg/dl)
	Women	44 - 80 µmol/l (0.5 - 0.9 mg/dl)
Urine:		8.84 - 13.3 mmol/24 hrs
		1 - 1.5 g/24 hrs

It is recommended that each laboratory establish its own reference range to reflect the age, sex, diet and geographical location of the population.

SPECIFIC PERFORMANCE CHARACTERISTICS

The following performance data were obtained using an Rx Monza analyser running at +37°C.

SERUM

LINEARITY

If the concentration exceeds 2168 µmol/l (24.5 mg/dl) in serum, dilute serum, 1+4 with 0.9% (w/v) NaCl and repeat the assay. Multiply the result by 5.

SENSITIVITY

The minimum detectable concentration of Creatinine with an acceptable level of precision was determined as 14.0 µmol/l (0.158 mg/dl).

PRECISION

Intra Assay

	Level 2	Level 3
Mean (mg/dl)	1.46	4.19
SD	0.040	0.068
CV (%)	2.75	1.63
n	20	20

Inter Assay

	Level 2	Level 3
Mean (mg/dl)	1.46	4.19
SD	0.042	0.122
CV (%)	2.86	2.91
n	20	20

CORRELATION

This method (Y) was compared with another commercially available method (X) and the following linear regression equation obtained:

Y = 1.098 X - 0.27
and a correlation coefficient of r = 0.9995

47 patient samples were analysed spanning the range 0.24 to 15.3 mg/dl.

URINE

LINEARITY

If the concentration exceeds 85309 µmol/l (963 mg/dl) in urine, dilute urine 1+4 with distilled water and repeat the assay. Multiply the result by 5.

SENSITIVITY

The minimum detectable concentration of Creatinine with an acceptable level of precision was determined as 2221 µmol/l (25.1 mg/dl).

REFERENCES

1. Bartels, H., Bohmer, M., (1972) Clin. Chem. Acta **37:** 193.
2. Schirmeister, J., H. Willmann, and H. Kiefer. (1964). Dtsch. Med. Wschr. **89:** 1018.

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