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CREATININE – Colorimetric

Reagent for the in-vitro quantitative determination of creatinine in human serum, plasma and urine on manual systems.

REF: BS.1/CC02.050.0100	100 test	REF: BS.1/CC02.125.0250	250 test
REF: BS.1/CC02.100.0200	200 test	REF: BS.1/CC02.250.0500	500 test

CLINICAL SIGNIFICANCE [1]

Creatine is synthesized in kidney, liver and pancreas It is transported in blood to muscles where it is phosphorylated to phosphocreatine. Creatinine is a waste product of creatine metabolism process of muscle contraction. The amount of creatinine produced each day is proportional to muscle mass. As creatinine is endogenously produced and released in body fluid at constant rate within narrow limits, therefore, measuring its clearance is indicative for glomerular filtration rate. Elevated serum creatinine levels are found in patients with renal malfunction, especially decreased glomerular filtration. Unlike urea, creatinine levels are unaffected by protein catabolism or other external factors, and hence a better indicator of renal function. Thus, serum creatinine is a significantly more reliable renal function screening test than serum urea.

METHOD PRINCIPLE [2-4]

Colorimetric method with deproteinization.
Creatinine reacts with picric acid in alkaline solution to form a coloured complex.

Creatinine + picrate —(**Alkaline pH**)→ **yellow-red complex**

REAGENT COMPOSITION

R1: Creatinine Standard	2 mg/dl (177µmol/L)
Reagent: **R2**: Picric acid **R3**: Sodium hydroxide	 38 mmol/L 1.6 mol/L

PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person. Do not ingest or inhale as reagent (R2) contains a low concentration of picric acid that its dry form is flammable and potentially explosive, hence it is recommended to avoid dryness of the material around the reagent bottle opening. reagent (R3) contains sodium hydroxide, that is an irritant (xi) chemical.

R36/38: Irritating to eyes and skin.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
  S56: dispose of this material and its container at hazardous or special waste collection point.
  S57: use appropriate container to avoid environmental contamination.
  S61: avoid release in environment.

For further information, refer to the Creatinine reagent material safety data sheet.

REAGENT PREPARATION, STORAGE AND STABILITY

BioScien Creatinine working solution is prepared by combining one volume of R2 with one volume of R3, e.g.1.0 ml R2 + 1.0 ml R3.
All reagents are stable until expiration date stated on label when properly stored refrigerated at 15-25°C. Working solution is stable for 5 hours at 15-25°C away from light.

Deterioration
The ***BioScien*** Creatinine reagent is normally clear, do not use reagent if it is turbid or if the absorbance of working solution is greater than 0.6 at 492 nm.

SPECIMEN COLLECTION AND PRESERVATION [5]

Serum or plasma
Specimen should be promptly separated from cells after blood collection. The only acceptable anticoagulants are heparin and EDTA. The biological half-life of creatinine in blood is few minutes and its stability is 7 day at 2-8°C and >1 year at -20°C.

Urine
Urine samples are diluted 1:50 with distilled water (dilute 1 part sample with 49 parts water) prior the assay, it is recommended to add thymol or toluene for urine preservation. Multiply result by 50 to compensate for dilution. Creatinine is stable in urine for 2 days at 15-25°C and 6 days at 2-8°C (freeze for longer storage).

SYSTEM PARAMETERS

Wavelength	546 nm (500 – 550 nm)
Optical path	1 cm
Assay type	End point
Direction	Increase
Sample Reagent Ratio e.g: Reagent volume Sample volume	1:1 1 ml 1 ml
Temperature	25˚C
Incubation time	20 minutes at 20 - 25˚C
Zero adjustment	Against air
Reagent Blank Limits	Low 0.3 AU High 0.7 AU
Sensitivity	0.4 mg/dL (0.035 mmol/L)
Linearity	15 mg/dL (1.32 mmol/L)

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes, automatic pipet
- Centrifuge and spectrophotometer

Deproteinization Procedure

Trichloroaceticacid (TCA)	1.0 ml
Specimen	1.0 ml

Mix will using glass rod to disperse the pricipitate. Cenrifuge at 3000 rpm for 10 minutes, then pour off the supernatant into clean tube. Stability: the supernatant is stable for 7 days at 2 - 4 ˚C.

ASSAY PROCEDURE

	Blank	*Standard*	*Specimen*	*Urine*
Distilled Water	0.5 ml	----	----	----
Standard	----	0.5 ml	----	----
TCA	0.5 ml	0.5 ml	----	0.5 ml
Supernatant	----	----	1.0 ml	----
Urine (1+ 49)	----	----	----	0.5 ml
Reagent mixture	1.0 ml	1.0 ml	1.0 ml	1.0 ml

Mix, and let stand for 20 minutes. at 20–25 ˚C. Measure the absorbance of specimen and standard against reagent blank at 546 nm.

CALCULATION

Serum or Plasma:

Creatinine concentration (mg/dl) = $\frac{\text{A specimen}}{\text{A standard}} \times 2$

Urine:

Creatinine concentration (mg/dl) = $\frac{\text{A specimen}}{\text{A standard}} \times 2 \times 50$

GFR by Creatinine clearance determination (ml/min):

Creatinine clearance (mg/dl) = $\frac{\text{UCr} \times \text{V}}{\text{SCr} \times 1440}$.

Correction for body surface area can be done using the following formula for creatinine clearance [6]***:***

Corrected Creatinine clearance (mg/dl) = $\frac{\text{UCr} \times \text{V} \times 1.73}{\text{SCr} \times \text{BSA}}$

Where:

UCr = Concentration of creatinine in urine (mg/dl)
SCr = Concentration of creatinine in serum (mg/dl)
V = Volume of urine flow in ml/min.
BSA = Body surface area in square meter
1.73 = Factor normalizes clearance for average body surface.

QUALITY CONTROL

To ensure adequate quality control, it is recommended that normal and abnormal commercial control serum of known concentrations included in each run. If control values are found outside the defined range, check the instrument calibration, and reagent for problems. If control still out of range please contact ***BioScien*** technical support.

PERFORMANCE CHARACTERISTICS

Precision	Within run (Repeatability)		Run to run (Reproducibility)	
	Normal level	High level	Normal level	High level
n	20	20	20	20
Mean mg/dl	1.55	4.58	1.67	4.63
SD. mg/dl	0.069	0.1	0.081	0.19
CV. %	4.45	2.2	4.58	2.7

The results of the performance characteristics depend on the analyzer used.

Accuracy (Methods Comparison)

Result obtained from ***BioScien*** Creatinine reagent compared with commercial reagent of the same methodology performed on 20 human sera give a correlation of 0.996.

Sensitivity

When run as recommended, the minimum detection limit of the assay is 0.4 mg/dL Creatinine (0.035 mmol/L).

Linearity

The reaction is linear up to Creatinine concentration of 15 mg/dl (1.32 mmol/L). Specimens showing higher concentration should be diluted 1+4 using physiological saline and repeat the assay (result×5).

INTERFERING SUBSTANCES [5]

Haemolysis

No significant interference from Erythrocyte contamination.

Icterus

Serum bilirubin levels higher than 5 mg/dL (85 µmol/L) decrease serum creatinine.

lipemia

Lipid may cause high absorbance flagging if present, dilute samples is recommended.

Others

Other drugs and substances may interfere.

EXPECTED VALUES [5]

Serum and plasma	***mg/dl***	***[µmol/L]***
Male	0.9-1.5	[80-133]
Female	0.7-1.3	[62-115]
Urine	***g/24hrs***	***[µmol/24hrs]***
Male	1.1-2.8	[124-230]
Female	0.9-1.6	[97-177]
GFR (Glomerular Filtration Rate)		***ml/min.***
Male		85-125
Female		75-115

DYNAMIC RANGE

0.4 - 15 mg/dl (0.035 – 1.32 mmol/L).

REFERENCES

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3. DI Giorgio J: Nonprotein nitrogenous constituents. In clinical chemistry – principles and technics, 2 nd ed. RJ Henry, DC Cannon, JW Winkelman, editors, Harper and Row, Hagerstown (MD), 1974, pp 541-553.
4. Heinegaard, D., and Tinderstrom, G. Clin. Chim. Acta. 43: 305 (1973).
5. Tietz N. W., Clinical Guide to Laboratory Tests, 4th Edition, (2006) p 316-321.
6. Tietz NW: Textbook of clinical chemistry. WB saunders, philadelphia, 1986, pp 1271- 1281.

SYMBOLS IN PRODUCT LABELLING

IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number		Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		

EC REP **Medical Device Safety Service**
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CE