SPECTRUM

The Creative Approach to Bioscience

Creatinine - Colorimetric

REF: 235 001	**2 x 100 ml**	**200 test**
REF: 235 002	**4 x 100 ml**	**400 test**
REF: 235 003	**8 x 100 ml**	**800 test**
REF: 235 004	**2 x 500 ml**	**1000 test**

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorised Representative		Use by/Expiration Date
IVD	For in-vitro diagnostic use		CAUTION. Consult instructions for use
LOT	Batch Code/Lot number		
REF	Catalogue Number		Manufactured by
	Consult instructions for use		(Xi) - Irritant
	Temperature Limitation		(C) - Corrosive

Intended Use

Spectrum Diagnostics creatinine reagent is intended for the in-vitro quantitative, diagnostic determination of creatinine in human serum or urine on manual system.

Background

Creatine is synthesized in kidney, liver and pancreas. It is transported in blood to other organs such as muscle and brain where it is phosphorylated to phosphocreatine. Some free creatine in muscle is converted to creatinine daily, and the amount of creatinine produced is proportional to muscle mass. In the absence of renal disease, excretion rate of creatinine in an individual is relatively constant. Therefore, measurement of creatinine clearance is useful in detecting renal disease and estimating the extent of impairment of renal function. Both serum creatinine and urea levels are elevated in patients with renal malfunction, especially decreased glomerular filtration. In the early stage of kidney damage, increase in serum urea level usually precedes the increase in serum creatinine .However serum urea levels may be affected by dehydration, diet and protein metabolism. On the other hand serum creatinine levels tend to be constant and unaffected by such factors. Thus serum creatinine is a significantly more reliable renal function screening test than serum urea.

Method

Colorimetric method with deproteinization.

Assay Principle

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

Creatinine + picrate $\xrightarrow{\text{NaOH}}$ yellow-red complex

Reagents

Standard creatinine (ST)
2 mg/dL 177 µmol/L

Reagent 1 (R1)
Picric acid 38 mmol/L
Reagent 1 contains a low concentration of picric acid, a chemical which, in its dry form, is flammable and potentially explosive. For this reason, it is recommended that drains be well flushed with water when discarding the reagent, spills be cleaned up at once, and dried material not be allowed to build up around the reagent bottle opening.
Irritant (Xi)
R38 Irritating to skin.
R41 Risk of serious damage to eyes.
S24/25 Avoid contact with skin and eyes.

Reagent 2 (R2)
Sodium hydroxide 1.6 mol/L
Reagent 2 contains caustic material.
Corrosive (C)
R35 cause severe burns.
R41 Risk of serious damage to eyes.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S28 After contact with skin, wash immediately with plenty of soap and water.

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

Additional Reagent

Trichloroacetic acid 1.2 mol/L.

Reagent Preparation

Prepare working solution as follows:
Combine one volume of R1 with one volume of R2,
e.g. 1.0 ml R1 + 1.0 ml R2

Precautions and Warnings

Do not ingest or inhalate. 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.

Reagent Storage and Stability

All reagents are stable until expiration date stated on label when stored at 15 - 25 ^{o}C.
Working solution is stable for 5 hours at 15 – 25 ^{o}C away from light.

Deterioration

The creatinine reagents are not suitable for use if combined reagents have an absorbance greater than 0.6 at 492 nm measured in a 1-cm light path or if the reagents develop a hazy appearance.

Specimen Collection and Preservation

Serum or plasma
Both are suitable for analysis. The only acceptable anticoagulants are heparin and EDTA. Specimen should be promptly separated from cells after blood collection. The biological half-life of creatinine in blood is few minutes.
Stability: 7 days 2 - 8 ^{o}C ; > 1 year at – 20 ^{o}C

Urine
Thymol or toluene may be used for urine preservation. To determine creatinine concentration in urine, dilute 1 part sample with 49 parts isotonic saline prior to assay. Multiply result by 50 to compensate for dilution.
Stability: 2 days at 15 - 25 ^{o}C ; 6 days at 2 - 8 ^{o}C
6 months at -20^{o}C away from light

System Parameters

Wavelength	546 nm (500 - 550 nm)
Optical path	1 cm
Assay type	End point
Direction	increase
Sample : Reagent Ratio	1 : 1
e.g.: Reagent volume	1 ml
Sample volume	1 ml
Temperature	25 ^{o}C
Zero adjustment	Against Air
Reagent Blank Limits	Low 0.30 AU
	High 0.6 AU
Sensitivity	0.4 mg/dL (0.035 mmol/L)
Linearity	15 mg/dL (1.32 mmol/L)

Deproteinization Procedure

Pipette into centrifuge tubes
Trichloroaceticacid (TCA) 1.0 ml
Serum or heparinaized plasma 1.0 ml
(TCA reagent is available upon request)

Mix well 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 ^{o}C.

Procedure

Pipette into test tubes

	Blank	Standard	Sample	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 ^{o}C. Measure the absorbance of specimen and standard against reagent blank at 546 nm.

Calculation

Concentration of creatinine in serum:

$$\text{Creatinine (mg/dL)} = \frac{\text{(Aspecimen)}}{\text{(Astandard)}} \times 2$$

Concentration of creatinine in urine:

$$\text{Creatinine (mg/dL)} = \frac{\text{(Aspecimen)}}{\text{(Astandard)}} \times 2 \times 50$$

Creatinine clearance:

$$\frac{\text{mg creatinine / dL urine x mL urine / 24 hours}}{\text{mg creatinine / dL serum x 1440}}$$

Correction for body surface area can be done using the following formula for creatinine clearance:

Serum creatinine / min. per standard surface area =

$$\frac{\text{UCr x V}}{\text{PCr}} \times \frac{1.73}{\text{A}}$$

Where: UCr = Concentration of creatinine in urine (mg/dL)
PCr = Concentration of creatinine in plasma (mg/dL)
V = Volume of urine flow in mL/min.
A = Body surface area in square meter .
1.73/A = Factor normalizes clearance for average body surface.

Note : Body surface area can be determined from height weight via normograms in Tietz [6].

Quality Control

Normal & abnormal commercial control serum of known concentrations should be analyzed with each run.

Methods Comparison

A comparison between Spectrum Diagnostics Creatinine colorimetric reagent and a commercial reagent of the same methodology was performed on 40 human sera. A correlation (R) of 0.996 was obtained.

Sensitivity

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

Linearity

The reaction is linear up to a creatinine concentration of 15 mg/dL; specimens showing higher concentration should be diluted 1+4 using physiological saline and repeat the assay (result × 5).

Interfering Substances

Serum, plasma

Haemolysis
Erythrocyte contamination doesn't elevate results.

Icterus
Serum bilirubin levels in the pathological range may interfere with the results.

Lipemia
Lipemic specimens may cause high absorbance flagging. Diluted sample treatment may be recommended.

Expected Values

Serum, plasma		
Females	0.7-1.3 mg/dL	62-115 µmol/L
Males	0.9-1.5 mg/dL	80-133 µmol/L
Urine(24 hrs)		
Females	0.9 – 1.6 g/24 hrs	
Males	1.1 – 2.8 g/24 hrs	
Creatinine clearance		
Females	75 – 115 mL / min	
Males	85 – 125 mL / min	

Spectrum Diagnostics does not interpret the results of a clinical laboratory procedure;interpretation of the results is considered the responsibility of qualified medical personnel. All indications of clinical significance are supported by literature references.

Dynamic Range

0.4 - 15 mg/dL (0.035 - 1.32 mmol/L).

Waste Disposal

This product is made to be used in professional laboratories.
Please consult local regulations for a correct 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. refer to special instructions/safety data sheets.

References

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2. 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.
3. Doolan PD, Alpen EL, Theil GB: A clinical appraisal of the plasma concentration and endogenous clearence of creatinine. AM J Med 32:65, 1962.
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5. Tobias GJ, Mclaughlin RF, Hopper J: Endogenous creatine clearence. N Engl J Med 266:317, 1962.
6. Tietz NW: Textbook of clinical chemistry. WB saunders, philadelphia, 1986, pp 1271- 1281.

ORDERING INFORMATION	
CATALOG NO.	**QUANTITY**
235 001	2 x 100 ml
235 002	4 x 100 ml
235 003	8 x 100 ml
235 004	2 x 500 ml

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ISO 13485:2016