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	Manufactured by
REF Catalogue Number	(Xi) - Irritant
Consult instructions for use	
Temperature Limitation	

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.

Method

Colorimetric method with deproteinization.

Assay Principle

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

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

Reagents

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

Reagent 1 **Irritant (Xi)**
Picric acid 38 mmol/L

The reagent 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.

Reagent 2 ***Corrosive (C)***

Sodium hydroxide 1.6 mol/L

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.

Additional Reagent

Trichloroacetic acid 1.2 mol/L.

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 Preparation , Storage and Stability

All reagents are stable till the expiration date stated on label when stored at 15 - 25 ^{O}C.Once opened, the reagent is stable for 6 months and the standard is stable for 3 months at the specified temperature.if contamination is avoided.
Working solution is prepared by adding equal volumes from R1 and R2. Working solution is stable for 5 hours at 15 – 25 away from light .

Deterioration

For invitro diagnostics use only .The creatinine reagents are not suitable for use if working solution 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

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:

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

Concentration of creatinine in urine:

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

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:

$$\text{Serum creatinine / min. per standard surface area} = \frac{UCr \times V}{PCr} \times \frac{1.73}{A}$$

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 and weight via normograms in Tietz [6].

Performance Characteristics

Precision

Within run (Repeatability)

	Level 1	Level 2
n	20	20
Mean (mg/dL)	1.37	4.32
SD	0.072	0.12
CV%	5.26	2.78

Run to run (Reproducibility)

	Level 1	Level 2
n	20	20
Mean (mg/dL)	1.59	4.38
SD	0.094	0.16
CV%	5.91	3.65

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).

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.

Interfering Substances

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	

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

1.Tietz NW: Textbook of clinical chemistry. WB saunders, philadelphia, 1986.
2..Spencer K, Price CP: A review of Non-enzyme mediated reaction and their application to centrifugal analyzers. IN centerfugal analyzers in clinical chemistry.
3.Tobias GJ, Mclaughlin RF, Hopper J: Endogenous creatine clearence,1962.

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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