AutoPure®

Sphera System Packs

Creatinine (Enzymatic)

Introduction

1. Autopure Creatinine (Enzymatic) is a reagent kit for quantitative determination of creatinine concentration in human serum and urine based on enzymatic method.
2. Autopure Creatinine (Enzymatic) is a ready-to-use, two liquid reagent system.
3. With Autopure Creatinine (Enzymatic), the assay is linear upto 200 mg/dL.

Clinical Significance

Creatinine is a chemical waste molecule that is generated from muscle metabolism. Creatinine is produced from creatine, a molecule of major importance for energy production in muscles. Approximately 2% of the body's creatine is converted to creatinine every day.

Creatinine is transported through the bloodstream to the kidneys. The kidneys filter out most of the creatinine and dispose it of in the urine. The kidneys maintain the blood creatinine in a normal range.

Creatinine has been found to be a fairly reliable indicator of kidney function. As the kidneys become impaired the creatinine level in the blood will rise. Abnormally high levels of creatinine thus warn of possible malfunction or failure of the kidneys.

Principle

Through a series of enzymatic reactions, creatinine is converted to glycine. Endogenous components such as creatine and sarcosine are eliminated in the first step of the reaction.The hydrogen peroxide formed reacts with TOOS in the presence of peroxidase (POD), to form a quinone dye.

The intensity of the colour is directly proportional to the concentration of creatinine in the specimen and is measured photometrically at 546 nm.

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

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

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

$$2H_2O_2 + \text{4-AAP} + \text{TOOS} \xrightarrow{\text{POD}} \text{Quinone dye} + 4H_2O$$

TOOS : N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine
4-AAP : 4-Aminoantipyrine

Reagent Storage, Stability & Handling

Autopure Creatinine (Enzymatic) is a ready-to-use, two liquid reagent system.

Shelf life
Stable till the expiry date indicated on the label, when stored at 2° - 8° C.

On-Board Reagent Stability

R1:28 days at 2°-8°C after opening
R2:28 days at 2°-8°C after opening

Protect the reagents from direct light and contamination. Do not freeze the reagent.

Components & Concentration of Working Solution

Component	Concentration
R1	
• Creatinase	> 15 KU/L
• Sarcosine oxidase (SOD)	> 1.2 KU/L
• TOOS	> 1 mmol
R2	
• Creatininase	> 30 KU/L
• Peroxidase	> 4 KU/L
• 4 – AAP	> 0.6 mmol

Specimen Collection & Preservation

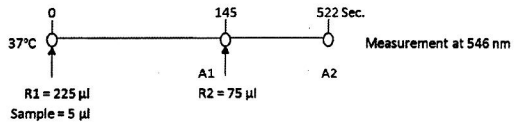
Collect sample using standard sampling tube. Serum is the specimen of choice.

Urine samples (24 hrs) should be diluted appropriately before use.

Creatinine in samples is stable for 24 hours if stored at 2°-8°C.

Procedure

Autopure Creatinine (Enzymatic) can be used on various automated analyzers. The procedure given below is for Sphera auto-analyzer.



Calculations

Fully automated system automatically calculates the Creatinine concentration of each sample.

Calibration

For calibration, it is recommended to use C.f.a.s.* or any other suitable calibrator material.

Calibration frequency
Re-calibration is recommended

- Whenever the reagent lot is changed.
- As per the requirement of quality control procedures.

*C.f.a.s. is brand name of Roche Diagnostics, Germany.

Quality Control

Each batch of Autopure Creatinine (Enzymatic) is assayed with atleast six controls sera - Precinorm**, Precipath**, Biorad I***, Biorad II***, Accutrol normal and Accutrol Abnormal prior to release▲.

To ensure adequate quality control, it is recommended that the laboratory should use a normal and an abnormal commercial reference control material. It should be realized that the use of quality control material checks both reagent and instrument functions together.
If the control values fall outside the specified limits, each of the below criteria should cross-checked and corrected:

- Proper instrument function – wavelength setting, light source and temperature control
- Cleanliness of probes and cuvettes.
- Bacterial contamination of wash water used by the instrument
- Expiry date of the reagent kit

**Brand name of Roche Diagnostics, Germany; ***Brand name of Biorad, USA; ▲subject to availability of control serum.

Expected Values

	Serum	Urine*
Men	0.7 - 1.2 mg/dL	40 - 278 mg/dL
Women	0.5 – 1.0 mg/dL	29 - 226 mg/dL

* First morning urine

Expected range varies from population to population. It is therefore recommended that each laboratory should establish its own reference range. For diagnostic purposes, creatinine results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Performance Characteristics

Linearity

With Autopure Creatinine (Enzymatic), the assay is linear upto 200 mg/dL. Determine samples with higher concentration via the rerun function. On instruments without rerun function, manually dilute the samples with higher concentration using normal saline (0.9% NaCl) or distilled/deionized water and repeat the assay. The result obtained should be then multiplied with dilution factor to obtain correct creatinine concentration.

Interferences

There is no significance interference in samples containing upto 1000 mg/dL Triglyceride and 40 mg/dL Bilirubin.

Precision Studies

Reproducibility was determined using three levels of serum specimen as shown below:

	Within run (n = 80)			Between run (n = 80)		
Serum	Mean (mg/dL)	SD (mg/dL)	CV %	Mean (mg/dL)	SD (mg/dL)	CV %
Sample 1	0.74	0.015	2.1	0.74	0.022	3.0
Sample 2	1.38	0.015	1.1	1.38	0.026	1.9
Sample 3	4.04	0.029	0.7	4.04	0.058	1.4

Co-Relation Studies

A comparison of Creatinine determination using Autopure Creatinine (Enzymatic) and reference competitor's product gave the following co-relation(mg/dL):

Linear Regression (Serum):

$y = 0.9467x + 0.0643$

$r^2 = 0.9981$

No. of samples measured: 55

References

1. Tietz, N. W. (Ed): Fundamentals of Clinical Chemistry, W. B. Saunders Co., Philadelphia, 865(1982).
2. Artiss, J.D., Mc Enroe, R.J., Zak, B.; Clin. Chem, 30 (1984)1389.
3. Mazzachi BC, Peake MJ, Ehrhardt V. Reference Range and Method Comparison Studies for Enzymatic and Jaffe Creatinine Assays in Plasma and Serum and Early Morning Urine. Clin. Lab 2000;46;53-55.

AR. No.: I 125 ASCE1-2016-10-001

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Application sheet