ENZYMATIC CREATININE SD

Instructions for use

Ref.: 167

Intended use . System for the determination of creatinine in serum and urine by final point reaction.

Professional use.

[For in vitro diagnostic use only.]

Test principle . The creatinine present in the sample is converted to creatine by the action of the enzyme creatinine amidohydrolase. The creatine produced is hydrolyzed to sarcosine and urea by the action of the enzyme creatine amidinohydrolase. Next, the enzyme sarcosine oxidase promotes the oxidative demethylation of sarcosine, leading to the production of glycine, formaldehyde and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide formed reacts with N-ethyl-N-sulfopropyl-m-toluidine (ESPMT) and 4-aminoantipyrine, producing a quinoneimine that has a maximum absorbance at 546 nm.
The color intensity of the reaction product is directly proportional to the creatinine concentration in the sample.

$$\text{Creatinine} + H_2O \xrightarrow{\text{Creatinine amidohydrolase}} \text{Creatina}$$

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

$$\text{Sarcosine} + H_2O + O_2 \xrightarrow{\text{Sarcosine oxidase}} \text{Glycine} + \text{Formaldehyde} + H_2O_2$$

$$2\,H_2O_2 + \text{ESPMT} + \text{4-aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4\,H2O$$

System features . Labtest's Enzymatic Creatinine SD Ref. 167 system uses the enzymes creatinine amidohydrolase, creatine amidinohydrolase and sarcosine oxidase in conjunction with the Trinder reaction, to determine the concentration of creatinine in serum, plasma and urine samples. The enzymatic methodology provides greater specificity to the determination of the analyte, eliminating the interference of plasma proteins and other chromogens, commonly observed with direct methods using the Jaffé reaction[1].

The calibrator material indicated is calibrated with SRM 914 from the National Institute of Standards and Technology (NIST) and makes the results traceable to the definitive IDMS method (isotopic dilution, mass spectrometry), meeting the recommendations of the National Kidney Disease Education Program (NKDEP) to standardize serum creatinine measurement[4].

Metodology . Enzymatic -Trinder

Reagents

1. R 1 **- Reagent 1 - Store at 2 - 8 ºC.**
Contains buffer pH 7.4, creatine amidinohydrolase ≤ 60 IU/mL, sarcosine oxidase ≤ 17 IU/mL, ascorbate oxidase ≤ 16 IU/mL and N-ethyl-N-(3-sulfopropyl)-3 m-toluidine ≤ 0,21 mg/mL.

2. R 2 **- Reagent 2 - Store at 2 - 8 ºC.**
Contains buffer pH 7.3, creatinine amidohydrolase ≤ 670 IU/mL, peroxidase ≤ 91 IU/mL and 4 aminoantipyrine ≤ 0.9 mg/mL and sodium azide < 0.1%.

The reagents must remain outside the storage temperature only for the time necessary to obtain the volume to be used. Avoid exposure to direct sunlight.

Unopened reagents, when stored under the indicated conditions, are stable until the expiration date printed on the label. During handling, reagents are subject to chemical and microbial contamination that can cause reduced stability.

Precautions and warnings

Usual safety precautions must be applied when handling reagents, which must not be pipetted by mouth. Care must be taken to avoid ingestion and in case of contact with eyes, wash immediately with large amounts of water and seek medical assistance.

Reagent 2 contains sodium azide which is toxic. Do not ingest and, in case of contact with eyes, wash immediately with plenty of water and seek medical help. Azide can form highly explosive compounds with lead and copper piping. Therefore, use large volumes of water to discard the reagents.

Materials required not provided

1. Analyzer capable of accurately measuring absorbance in 546 nm (540 to 550nm).

2. Calibrator – Calibra H Labtest series.

3. Pipettes to measure reagentes and samples.

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Specimen collection and preparation

A Standard Operating Procedure (SOP) must be created for sample collection, preparation and storage. We emphasize that errors due to the sample can be much greater than errors occurring during the analytical procedure.

Serum. The analyte is stable for 7 days at 2 - 8 ºC.

24-hour urine must be centrifuged. The urine sample must not receive preservatives or conservatives and must be refrigerated during the collection period and after it is received at the laboratory.

As no known test can ensure that samples of human biological material do not transmit infections, all samples must be considered as potentially infectious. Therefore, when handling them, established biosafety standards must be followed.

To dispose of reagents and biological material, we suggest applying local, state or federal environmental protection standards.

Interference

Chyle concentrations up to 1000 mg/dL, bilirubin (free and conjugated) up to 20 mg/dL, hemoglobin up to 500 mg/dL, ascorbic acid up to 100 mg/dL, creatine up to 10 mg/dL, L-proline up to 10 mg/dL do not significantly interfere with the reaction.

Samples with bilirubin, hemoglobin and triglycerides in concentrations greater than those mentioned above must be diluted in NaCl 150 mmol/L (0.85%) before carrying out the tests. Multiply the result obtained by the dilution factor.

Samples containing azide may present inaccurate results for creatinine concentration, caused by insufficient creatine conversion.

To evaluate the approximate concentration of hemoglobin in a sample, proceed as follows: dilute 0.05 mL of the sample in 2.0 mL of 150 mmol/L NaCl (0.85%) and measure the absorbance at 405 or 415 nm, setting the zero with deionized or distilled water.

$$\text{Hemoglobin (mg/dL)} \approx \text{Absorbance}_{405} \times 601$$
$$\text{Hemoglobin (mg/dL)} \approx \text{Absorbance}_{415} \times 467$$

Procedure

To measure creatinine in urine, dilute the sample 1:5 (0.2mL of urine + 0.8mL of 150 mmol/L NaCl). Multiply the result obtained by 5.

Primary wavelength: 546 nm
Secondary wavelength: 800 nm
Temperature: 37 ºC
Volume of R1*: 195 µL
Sample volume*: 5.5 µL
Reading 1 (Absorbance 1): After 300 seconds of incubation at 37 ºC of R1 + sample
Volume of R2*: 65 µL
Reading 2 (Absorbance 2): After 300 seconds of incubation at 37 ºC of R1 + sample + R2

*Sample and reagent volumes can be modified proportionally without compromising test performance. In case of volume reduction, it is essential that the minimum volume necessary for photometric measurement is observed.

Calibration. Automated systems

2-point Calibration
Point 0: Reagent blank – deionized water or NaCl 150 mmol/L (0,85%);
Point 1: Calibrator – Calibra H Labtest series.

The creatinine concentration in Calibra H is traceable to the National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 914.

Calibration frequency
When internal quality control indicates.
When using a new batch of reagent.
When using new reagent bottles from the same lot, if a new calibration was performed while using the previous bottle.

Calculation. According to NKDEP[4] recommendations, results should be reported with two decimal places to avoid systematic errors caused by rounding, which can reach ±6%.

Δabs of Test or Calibrator = Abs 2 - Abs 1

$$\text{Creatinine (mg/dL)} = \frac{\Delta\text{Abs Test}}{\Delta\text{Abs Calibrator}} \times \text{Calibrator conc. mg/dL}$$

Urine Creatinin

$$\text{Urine Creatinine (mg/24 h)} = \frac{\text{Urine Creatinine (mg/dL)}}{100} \times \text{Urine volume (mL/24 h)}$$

mg/kg weight = mg/24 hours divided by body weight.

Endogenous creatinine clearance. Instruct the patient to correctly collect 24-hour urine.

Measure creatinine in serum and urine. Serum can be obtained at any time during the urine collection period.

Apply the results obtained to the equation below:

$$\text{Clearance} = \frac{U}{S} \times MV \text{ (mL/minute)}$$

U: urine creatinine (mg/dL)
S: serum creatinine (mg/dL)
MV: minute volume (24-hour urinary volume, in mL, divided by 1440).

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Note.: The clearance must be corrected for the patient's body surface, which is obtained through the nomogram correlating weight and height, or using the equation below:

$A = W^{0,425} x H^{0,725} x 0,007184$

A = body surface area (m^2)
W = weight (kg)
H = height (cm)

Multiply the clearance value by 1.73 and divide by the patient's body surface.

Glomerular filtration rate . The NKDEP[4] strongly recommends that laboratories report the estimated glomerular filtration rate (eGFR) in all reports containing creatinine results (see Clinical Significance).

When plasma creatinine results are traceable to the IDMS method, the following equations are used that apply creatinine (CREA), age (18 to 70 years) and sex.

Women
$eGRF\ (mL/min/1,73m^2) = 175 * (CREA)^{-1,1154} * (Age)^{-0,203} * 0,742$

Men
$eGRF\ (mL/min/1,73m^2) = 175 * (CREA)^{-1,1154} * (Age)^{-0,203}$

According to NKDEP[4] recommendations, eGFR must be reported as a calculated value when the result is equal to or less than 60 $mL/min/1.73m^2$. When the calculated value is higher than 60, it must be reported as follows: Heigher than 60 $mL/min/1.73m^2$ or $>60\ mL/min/1.73m^2$.

Operating interval . The reaction is linear between 0.0 mg/dL and 100 mg/dL. For higher values, dilute the sample with 150 mmol/L NaCl (0.85%) and repeat the determination. Multiply the result obtained by the dilution factor.

Internal quality control . The laboratory must maintain an internal quality control program that clearly defines applicable regulations, objectives, procedures, criteria for quality specifications and tolerance limits, corrective actions and recording of activities. Control materials should be used to monitor measurement inaccuracy and calibration deviations. It is recommended to use products from the Qualitrol H - Labtest line for internal quality control in clinical chemistry tests. It is suggested to try to meet the specifications proposed by NKDEP[4] for the coefficient of variation ≤ 4% and systematic error (bias) ≤ 5%.

Expected values . The ranges should be used as a guide only[5,6,7]. It is recommended that each laboratory establishes its own reference range for the population served.

	Serum (mg/dL)*
Newborn	0.31 - 0.92
2 weeks - 1 year	0.16 - 0.39
1 - < 3 years	0.17 - 0.35
3 - < 5 years	0.26 - 0.42
5 - < 7 years	0.29 - 0.48
7 - < 9 years	0.34 - 0.55
9 - < 11 years	0.32 - 0.64
11 - < 13 years	0.42 - 0.71
13 - < 15 years	0.46 - 0.81
Adults (women) 18 - 74 years	0.53 - 1.00
Adults (men) 18 - 74 years	0.70 - 1.20

* Ranges established for results traceable to the IDMS method.

There are no established intervals for the 15 and 18 age group. It is suggested to use the ranges for adult women and men.

Conversion of mg/dL to SI units: μmol/L = mg/dL x 88.4

	Urine (mg/Kg/24 hours)
2 - 3 years	6 - 22
> 3 years	12 - 30
Adults (women)	16 - 22
Adults (men)	21 - 26

	Creatinine clearance ($mL/min/1.73m^2$)**
Children	70 - 140
Adults (women)	88 - 128
Adults (men)	97 - 137

** Intervals no established for results traceable to the IDMS method.

NKDEP[4] recommends calculating the glomerular filtration rate (eGFR) instead of Creatinine Clearance, using the creatinine result traceable to the IDMS method.

Peformance characteristics[8]

Method comparison . The Enzymatic Creatinine method was compared with another enzymatic method, obtaining the following results and observing:

For serum samples:

	Comparative method	Enzymatic Creatinine SD
Sample nature	Serum	
Sample number	59	
Concentration interval (mg/dL)	0.52 - 1.28	0.53 - 1.30
Estimate mean (mg/dL)	0.81	0.84
Regression equation	Enzymatic Creatinine SD = 1.0107* Comparative - 0.0094	
Correlation coefficient	0.9926	

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Using the regression equation, the following total errors were found for the Enzymatic Creatinine SD method:

Decision levels for creatinine evaluation	Creatinine estimated using the regression equation	Systematic erros estimated based on creatinine decision levels
mg/dL	mg/dL	%
1.00	1.00	1.98
1.20	1.20	1.54
2.00	2.00	1.85

The total error obtained is smaller than the total error of the minimum specification (11.4%)[1].

For urine samples:

	Comparative method	Enzymatic Creatinine SD
Sample nature	Serum	
Sample number	40	
Concentration interval (mg/dL)	17.06 – 112.56	16.86 – 114.50
Estimate mean (mg/dL)	58.98	58.32
Regression equation	Enzymatic Creatinine SD = 1.0019* Comparative – 0.9983	
Correlation coefficient	0.9983	

Using the regression equation, the following total errors were found for the Enzymatic Creatinine SD method:

Decision levels for creatinine evaluation	Creatinine estimated using the regression equation	Systematic erros estimated based on creatinine decision levels
mg/dL	mg/dL	%
22	21.94	2.11
30	29.67	2.36
100	97.28	3.98

The total error obtained is smaller than the total error of the minimum specification (11.4%)[1].

Imprecision

Imprecision – Within Run

	N	Mean	SD	CV%
Sample 1	20	1.07	0.0093	0.88
Sample 2		4.47	0.0281	0.63

Imprecision – Run-to-Run

	N	Mean	SD	CV%
Sample 1	25	1.04	0.012	1.12
Sample 2		4.34	0.033	0.76

The results indicate that the method meets the minimum specification for CV ($\leq 3.2\%$)[1].

Methodology sensitivity . A sample containing no creatinine was used to calculate the detection limit of the assay, and a value equal to 0.115 mg/dL was found, equivalent to the mean of 5 assays plus two standard deviations.

Effect of matrix dilution . A sample with a value equal to 81.7 mg/dL was used to evaluate the system's response to matrix dilutions with 150 mmol/L NaCl (0.85%). Using dilution factors ranging from 1.25 to 20, a recovery of 8% was found.

Summary[1,2,3] . The constancy in formation and excretion makes creatinine a very useful marker of renal function, especially glomerular filtration, due to its relative independence from factors such a diet, degree of hydration and protein metabolism. Therefore, determining plasma creatinine is a safer renal function test than urea.

Creatinine should not be used alone to evaluate the glomerular filtration rate or detect the presence of chronic kidney disease because it is affected by the glomerular filtration rate and by independent factors such as age, sex, race, diet, muscle mass, drugs and methods laboratory analytics.

More precise and accurate estimates of eGFR can be obtained with equations that empirically combine all the average effects of factors that affect creatinine with the exception of glomerular filtration itself.

The currently recommended equation was developed from the Modification of Diet Renal Disease (MDRD) study using iothalamate clearance as the reference method, and provides results normalized to the standard body surface area 1.73m^2 (see Glomerular Filtration Rate).

The MDRD equation should only be used in individuals over 18 years of age and has not been evaluated in the following situations: individuals over 70 years of age, pregnant women, people with serious morbidities, individuals with extremes in body mass or muscle mass or with nutritional status strongly committed.

The National Kidney Desease Educational Program (NKDEP) developed a document that provides information that can help laboratories with the following points[4]:

1. Report accurate glomerular filtration estimation results based on serum creatinine measurement;

2. Understand NKDEP initiatives to standardize serum creatinine measurements;

3. Communicate appropriately to healthcare providers about the implications for changes in serum creatinine results that will result from standardization initiatives in creatinine measurement.

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Notes

1. Proper cleaning and drying of the material used are fundamental factors for the stability of the reagents and obtaining correct results.

2. The clinical laboratory aims to provide accurate and precise results. The use of water of inadequate quality is a potential cause of analytical errors. The water used in the laboratory must be of the appropriate quality for each application. Therefore, to prepare reagents, use in measurements and for use in the final rinse of glassware, water must have resistivity ≥1 megaohm.cm or conductivity ≤ 1 microsiemens/cm and silicate concentration < 0.1 mg/L.
When the deionizing column is saturated, several ions, silicates and substances with great oxidizing or reducing power are released, which deteriorate the reagents in a few days or even hours, altering the results in an unpredictable way. Therefore, it is essential to establish a water quality control program.

References

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8. Labtest: Date on file.

Presentation

Product	Reference	Contents	
Enzymatic Creatinine SD	167-2/72	R 1	2 X 54 mL
		R 2	1 X 18 mL
Enzymatic Creatinine SD CS series	167-2/72	R 1	2 X 54 mL
		R 2	2 X 18 mL
Enzymatic Creatinine SD Audmax i series	167-2/48	R 1	2 X 36 mL
		R 2	2 X 12 mL

For information about other commercial presentations, consult the website www.labtest.com.br or contact SAC.

Applications for automatic and semi-automatic systems are available.

Customer information

[Warranty conditions]

Labtest Diagnóstica guarantees the performance of this product within specifications until the expiration date indicated on the labels, provided that the precautions for use and storage indicated on the labels and in these instructions are followed correctly.

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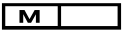
Símbolos utilizados com produtos diagnósticos in vitro

Símbolos usados con productos diagnósticos in vitro

Symbols used with IVD devices

	**Conteúdo suficiente para < n > ensaios** Contenido suficiente para < n > ensayos Sufficient content for < n > trials		**Risco biológico** Riesgo biológico Biological risk
	**Prazo de validade (aaaa-mm-dd ou mm/aaaa)** Fecha de expiración (aaaa-mm-dd o mm/aaaa) Expiration date (yyyy-mm-dd or mm/yyyy)		**Corrosivo** Corrosivo Corrosive
	**Limite de temperatura (conservar a)** Temperatura limite (conservar a) Temperature limit (store at)		**Tóxico** Tóxico Poison
	**Representante Autorizado na Comunidade Europeia** Representante autorizado en la Comunidad Europea Authorized Representative in the European Community		**Marca CE** Marcado CE CE Mark
	**Carcinogênico/mutagênico e/ou sensibilizante à respiração** Carcinogénico/mutagénico y/o sensibilizante respiratorio Carcinogenic/mutagenic and/or respiratory sensitizer		**Atenção** Atención Attention
	**Tóxico para os organismos aquáticos** Tóxico para los organismos acuáticos Toxic for aquatic organisms		**Data de fabricação** Fecha de fabricación Date of manufacture
	**Gases/líquidos comburentes** Gases/liquidos oxidantes Oxidizing gases/liquids		**Fabricante** Fabricante Manufacturer
	**Substância inflamável** Sustancia inflamable Flammable substance		**Uso veterinário** Uso veterinario Veterinary use
	**Período após abertura** Período post-abertura Period after-opening		**Liofilizado** Liofilizado Lyophilized
	**Produto de uso único** Producto de un solo uso Single use product		**Número do lote** Denominación de lote Batch code
	**Consultar instruções de uso** Consultar instrucciones de uso Consult instructions for use		**Número do catálogo** Número de catálogo Catalog Number
	**Instalar até** Instalar hasta Install before		**Controle** Control Control
	Material Calibrador/Padrão Material Calibrador/Estándar Calibrator/Standard Material		**Controle negativo** Control negativo Negative control
	Dispositivo médico de diagnóstico in vitro Dispositivo médico para diagnóstico in vitro In vitro diagnostic medical device		**Controle positivo** Control positivo Positive control
	**Reagente contendo microparticulas** Reactivo con microparticulas Reagent with microparticles		**Reagente** Reactivo Reagent
	Identificador único do dispositivo Identificador único del dispositivo Unique device identifier		**Pré-Tratamento** Pretratamiento Pre-Treatment

Ref.: 160125

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