CREATININE K
Insert

Ref.: 96

Intended use . System for creatinine determination in serum, plasma, urine and amniotic liquid by two point kinetic method.

Professional use.

[For in vitro diagnostic use only]

Test principle . Creatinine reacts to alkaline picrate yielding a red complex. The amount of the color is proportional to creatinine concentration (not corrected) in the sample.

Creatinine + Alkaline picrate ⟶ Creatinine picrate

Summary . Creatinine K applies a two point optimized procedure in order to improve the method specificity and minimize the susceptibility to interferences[1-3].

The measurement procedure is calibrated with the NIST SRM 914 and renders the results traceable to the IDMS (isotropic dilution, mass spectrometry) definitive method, which complies the National Kidney Disease Education Program (NKDEP) recommendations for standardization of serum creatinine measurement[4].

All the methods that apply the Jaffe reaction are susceptible to a constant systematic error, due to the plasmatic proteins ant other chromogens interference. In order to minimize this error and increase the Creatinine K results accuracy, Labtest recommends the use of the correction index[5,12] (see Calculation) that must be applied whatever are the found results.

Creatinine K presents a sample treatment procedure with ferricyanide[6,7] that oxides the present bilirubin (in concentration of 5.0 up to 19 mg/dL) and excludes its negative interference, while the desproteinization procedure removes interference of lipemic samples equivalent to a triglycerides value of 900 up to 1800 mg/dL[12]. This method was based on the performed studies that demonstrated that is possible minimize significantly the interferences caused by icteric and lipemic samples in creatinine measurement[3].

The alkaline picrate keeps the analytic performance when tightly closed at 2-8ºC for 15 days, allowing the preparation of a high volume of working reagent according to the laboratory routine.

The measurement procedure is applied in automated and semi-automated systems able to perform accurate measure of absorbance at 510 nm.

Methodology . Labtest

Reagents

1. R 1 - NaOH - Store at 15-30ºC.
Reagent label bears expiration date. Sodium hydroxide (200 mmol/L). Corrosive reagent.

2. R 2 - Picric Acid - Store at 15-30ºC.
Reagent label bears expiration date. Picric acid (22.2 mmol/L).

3. CAL - Standard 4.0 mg/dL - Store at 2-30ºC.
Reagent label bears expiration date. Creatinine 4.0 mg/dL.

4. R 4 - Ferricyanide - Store at 15-30ºC.
Potassium ferricyanide (11 mmol/L). Do not keep in the refrigerator.

Precautions and warnings

Disposal of all waste material should be in accordance with local guidelines.

The usual security cares should be applied on the reagent handling.

The NaOH (nº 1) is corrosive and may result in skin and eyes burns and irritations and ulcerations when ingested. In case of ingestion, immediately ingest a lot of water with lemon juice or vinegar. Do not induce vomiting and get medical assistance. In case of eyes contact, immediately flush eyes with plenty of water and get medical assistance.

In case of ingestion of Picric Acid (Nº 2), offer 4 glasses of water and if the individual is conscious, induce vomiting and get medical assistance.

Storage and stability . Unopened reagents, when stored at indicated temperature, are stable up to expiration date shown on the label. In order to avoid evaporation of the Standard, keep the bottle tightly closed.

Deterioration . Microbial or chemical contamination may decrease reagents stability.
Alkaline Picrate is not suitable for use if it has an absorbance over 0.200 at 510 nm when measured versus water as reference.

Sample

Use serum or plasma (fluoride, heparin, EDTA, oxalate and citrate). Creatinine is reportedly stable for about 7 days at 2-8ºC. The use of the anticoagulant Glistab (Labtest Ref.: 29) allows the collection of only one sample to the urea, glucose and creatinine measurements.

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24 hours urine and amniotic liquid must be centrifuged. The urine sample should not receive preservatives and must be kept at 2 - 8 ºC during the collection period and after the delivery in the laboratory.

No known test method can offer complete assurance that human blood samples will not transmit infectious diseases. Therefore, all blood derivatives should be considered potentially infectious.

Interference

Proteins present in the samples yield a positive interference introducing a constant systematic error. This error may be minimized applying a correction index. Since the urine has no proteins that may interfere, the correction index is not applied to the calculation of concentration in samples of urine. See applications of correction index on Calculation.

Creatinine determination in urine may be affected by the action of high amount of reducers substances present in cases of ketoacidosis. Boiling the urine sample for one minute eliminates partially these substances interference. The remaining interference is excluded in the kinetic measurement.

Bilirubin over 5 mg/dL interferes negatively in the reaction. Hemoglobin up to 180 mg/dL and triglycerides up to 900 mg/dL do not interfere in the reaction.

Eliminating the interferences action . Samples with bilirubin concentration of 5 to 19 mg/dL, the interference may be eliminated by the following procedure: add 0.05 mL of Ferricyanide (Nº 4) to 0.5 mL of the sample. Mix and wait 5 minutes. Determine the creatinine, multiply the result by 1.1 and apply the correction index.

If bilirubin concentration is over 19 mg/dL and lower than 38 mg/dL, dilute the sample1:2 with 0.85% NaCl (150 mmol/L). Add 0.05 mL of Ferricyanide (Nº 4) to 0.5 mL of the diluted sample. Mix and wait 5 minutes. Determine the creatinine, multiply the result by 2.2 and apply the correction index.

If triglycerides concentrations are ranging from 900 mg/dL and 1800 mg/dL the interference due the lipemic sample may be eliminated by the desproteinization procedure. If triglycerides concentrations are over 1800 mg/dL and lower than 3500 mg/dL, dilute the sample 1:2 with 0.85% NaCl (150 mmol/L), and follow the procedures with desproteinization for creatinine determination and multiply the result by 2. **Do not apply the correction index when using the desproteinization procedure.**

Dilutions over 1:2 are not recommended because, in samples with low creatinine concentrations, it is obtained results with significant errors due the increasing of the analytical imprecision.

Materials required not provided

1. Photometer with cuvette at 37ºC capable of measuring absorbance in kinetic method.
2. Automated equipment able to process one or two reagents (for automated applications).
3. Pipettes to measure reagents and samples.
4. Timer (for manual applications).

Preparing alkaline picrate

Mix 4 volumes of NaOH (Nº 1) with 1 volume of Picric Acid (Nº 2). Stable 15 days at 2-8ºC in a plastic bottle tightly closed. Deterioration is indicated by an absorbance over 0.200 if Alkaline Picrate is measured against water at 510 nm.

Atmospheric CO_2 alters significantly the NaOH (Nº 1) and Alkaline Picrate stability, if kept in open bottles. The stability modification is influenced by exposure time and environment conditions. It is suggested to keep in the equipment only the volume enough to the samples measurement or using the information of Quality Control as indicator for performing new calibration.

Procedure

Urine: dilute the sample 1:25 (0.2 mL of urine + 4.8 mL of distilled or deionized water). Multiply the result by 25.

Temperature control is absolutely important for reproducibility of results. Since the time of reaction is very small, it is necessary to use equipment with cuvette at 37ºC.

It is fundamental that the operations with samples and standards are performed always in the same way, keeping constant the time interval between the sample or standard mixture with the reagent and the beginning of the photometric measurement.

In case of the proposed volumes are not enough to the equipment for photometric readings, increase proportionally the volumes of Alkaline Picrate and sample or Standard.

Direct procedure . Zero the photometer at 510 nm (490 to 520 nm) with water. Add 0.10 mL of Standard or sample to 1.0 mL of Alkaline Picrate. Mix and insert into the cuvette. Start the timer and measure the absorbance after 30 and 90 seconds.

Procedure with desproteinization . Mix 0.2 mL of serum to 0.4 mL of Picric Acid (Nº 2), mix and centrifuge during 10 minutes. Adjust the zero of photometer at 510 nm (490 to 520 nm) with water. In other tube, pipette 0.8 mL of NaOH (Nº 1) and add 0.3 mL of the clear supernatant. Mix and insert into the cuvette. Start the timer and measure the absorbance after 30 and 90 seconds. The Standard should be measured using the direct procedure. Do not apply the correction index.

Calibration

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

Manual calibrations
Perform a new calibration after reagent lot change or when the internal quality control indicates.

Automatic Systems
Blank of reagents: water or 0.85% NaCl;
Standards: Calibra Series (Labtest calibrator for automated systems), which are traceable to NIST SRM 914.

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Calibration frequency
Three point calibration after reagent lot change;
Three point calibration when the internal quality control indicates.

Quality control . Interval must be adapted to the laboratory requirements. Each laboratory should establish corrective actions to be taken if values fall outside the control limits.

It is recommended to products from Qualitrol line - Labtest for internal quality control in clinical chemistry trials.

Calculations

$$\Delta A \text{ of Unknwon or Standard} = A_{90\ seconds} - A_{30\ seconds}$$

$$\text{Creatinine (not corrected)} = \frac{\Delta A_{unknown}}{\Delta A_{standard}} \times 4\ mg/dL$$

According with NCEP[4] recommendations, the results should be reported with two places of decimals in order to avoid systematic errors due to making the results round, which may reach ±6%.

Applying the correction index . Plasmatic proteins interference that occurs in Jaffe[5] reaction, introduce a constant error in the measurement which is minimized by the correction index (0.25 mg/dL). **The obtained results with the calibration and the correction are traceable to IDMS method and comply with the NKDEP[4] recommendations.**

Creatinine (corrected) = **Creatinine** (not corrected) - **Correction index** (0.25 mg/dL)

The use of correction index in automated measurements is presented in the Labtest products for automated equipments.

Urinary Creatinine

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

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

Since urine samples do not have proteins in concentrations which could induce constant errors, it is not necessary to apply the correction index.

Endogenous creatinine depuration . The patient should receive explanations in order to collect the 24 hours urine correctly.

Measure the serum and urine creatinine using the proposed methodologies. Serum may be obtained in any moment during the urine collection.

Apply the results obtained in the following equation:

$$\text{Depuration} = \frac{U}{S} \times VM\ (mL/minute)$$

U = creatinine in the urine (mg/dL)
S = corrected creatinine in the serum (mg/dL)
VM = Volume per minute (urinary volume of 24 hours, in mL, divided by 1440).

PS: Depuration must be corrected for the body surface of the patient that is obtained by a nomogram correlating weight and height, or using the following equation:

$$A = W^{0.425} \times H^{0.725} \times 0.007184$$

A = body surface (m^2)

W = weight (kg)
H = height (cm)

Multiply the depuration value by 1.73 and divide by the patient body surface.

Glomerular de filtration rate . The NKDEP[4] recommends that the laboratories report the estimated glomerular filtration rate (eGFR) in all the reports containing creatinine results.
When the plasmatic creatinine results are corrected and traceable to the IDMS, the following equations that apply creatinine (CREA), age (18 - 70 years) and sex are used.

Women
$$\mathbf{eGFR\ (mL/min/1.73m^2) = 175 * (CREA)^{-1.154} * (Age)^{-0.203} * 0.742}$$

Men
$$\mathbf{eGFR\ (mL/min/1.73m^2) = 175 * (CREA)^{-1.154} * (Age)^{-0.203}}$$

According NKDEP[4] recommendations, the eGFR must be reported as the calculated value when the result is ≤60 mL/min/1.73m^2. When the calculated value is >60, must be reported as: higher than 60 mL/min/1.73m^2 or >60 mL/min/1.73m^2.

Measurement/reportable range

The reaction is linear between 0.2 mg/dL and 12 mg/dL.

If creatinine concentration exceeds 12 mg/dL, the sample must be diluted with 0.85% NaCl. Multiply the result by the appropriate dilution factor and apply for correction index.

Expected range . Each laboratory should evaluate the transferability of the expected range to its own patient population and, if necessary, estimate its own reference range[5,10,11].

Serum/Plasma (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	0.53 - 1.00
Adults (men) 18 - 74	0.70 - 1.20

* Corrected values with the correction index
There isn't established range for the age 15 - <18 years. It's suggested to use the women and men adult range.

Conversion mg/dL to IS 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 Depuration (mL/minute/1,73 m^2)**

Children	70 - 140
Adults (women)	88 - 128
Adults (men)	97 - 137

** Established values for not corrected and not IDMS traceable results

The NKDEP[4] recommends calculate the glomerular filtration rate (eGFR) in substitution of Creatinine Depuration, using the creatinine IDMS traceable result after application of the correction index.

Performance characteristics[12]

Recovery studies . In two samples with creatinine concentrations of 2.7 and 8.7 mg/dL were added different quantities of the analyte. Subsequent analyses provided recoveries ranging from 97 to 100%. The mean proportional systematic error at 1.6 mg/dL decision level was 0.0232 mg/dL or 1.45%.

Method comparison . A group of 20 sera were assayed by the proposed method and an enzymatic method (traceable to IDMS method). Serum creatinine values ranged from 0.52 - 11.0 mg/dL. The comparisons yielded a correlation coefficient of 0.998 and regression equation was y = 1.0073x + 0.0281. The mean total systematic error (proportional and constant) at 1.00 mg/dL, 1.20 mg/dL and 2.00 mg/dL decision levels were 0.035 mg/dL (3.53%), 0.037 mg/dL (3.07%) and 0.043 mg/dL (2.13%), respectively.

Imprecision - Within Run

	N	Mean	SD	(%) CV
Sample 1	20	0.66	0.013	1.97
Sample 2	20	2.04	0.016	0.78
Sample 3	20	7.49	0.144	1.92

Imprecision - Run-to-Run

	N	Mean	SD	(%) CV
Sample 1	20	0.66	0.027	4.10
Sample 2	20	2.04	0.038	1.86
Sample 3	20	7.49	0.273	3.64

Analytical sensitivity . Detection limit: 0.14 mg/dL. The detection limit represents the lowest measurable creatinine concentration that can be distinguished from zero. It is calculated as two standard deviations of 20 replicates of one sample without creatinine.

Effects of matrix dilution . Two samples with values equal to 1.3 and 1.5 mg/dL were used to evaluate the system response in the matrix dilutions with NaCl 150 mmol/L (0.85%). Using dilution factors ranging from 2 to 16, obtained recoveries between 80 and 104%.

Notes

1. The material cleaning and drying are fundamental factors to the reagent stability and to obtain correct results.

2. The deionized or distilled water in the laboratory to prepare reagents, use in the measurements and for final glass washing must have resistivity ≥1 megaohm.cm, or conductivity ≤1 microsiems/cm and silicates concentration must be <0.1mg/L.

3. It is suggested to consult www.fxol.org in order to review physiopathological source and drugs interference in results and methodology.

References

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Presentation

Product	Reference	Contents	
Creatinine K	96-300	R 1	1 X 240 mL
		R 2	1 X 60 mL
		CAL	1 X 5 mL
		R 4	1 X 5 mL
Creatinine K Labmax 560/400	96-10/21,5	R 1	10 X 17 mL
		R 2	10 X 4,5 mL
		CAL	1 X 5 mL
		R 4	1 X 5 mL

The number of tests in automated systems **depends of the programmed parameters.**

Application procedures using Creatinine K are available for various automated systems.

Some presentations may be available after consult.

Customer information

[Warranty conditions]

Labtest Diagnóstica warrants the performance of this product under the specifications until the expiration date shown in the label since the application procedures and storage conditions, indicated on the label and in this insert, have been followed correctly.

CE

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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 > testes** Contenido suficiente para < n > tests Contains sufficient for < n > tests		**Risco biológico** Riesgo biológico Biological risk
	**Data limite de utilização (aaaa-mm-dd ou mm/aaaa)** Estable hasta (aaaa-mm-dd o mm/aaaa) Use by (yyyy-mm-dd or mm/yyyy)		**Marca CE** Marcado CE CE Mark
	**Material Calibrador** Material Calibrador Calibrator Material		**Tóxico** Tóxico Poison
	**Material Calibrador** Material Calibrador Calibrator Material		**Reagente** Reactivo Reagent
	**Limite de temperatura (conservar a)** Temperatura limite (conservar a) Temperature limitation (store at)		**Fabricado por** Elaborado por Manufactured by
	**Representante Autorizado na Comunidade Europeia** Representante autorizado en la Comunidad Europea Authorized Representative in the European Community		**Número do lote** Denominación de lote Batch code
	**Consultar instruções de uso** Consultar instrucciones de uso Consult instructions for use		**Controle** Control Control
	**Número do catálogo** Número de catálogo Catalog Number		**Controle negativo** Control negativo Negative control
	**Adições ou alterações significativas** Cambios o suplementos significativos Significant additions or changes		**Controle positivo** Control positivo Positive control
	**Produto diagnóstico in vitro** Dispositivo de diagnóstico in vitro In vitro diagnostic device		**Controle** Control Control
	**Liofilizado** Liofilizado Lyophilized		**Corrosivo** Corrosivo Corrosive
	**Período após abertura** Período post-abertura Period after-opening		**Uso veterinário** Uso veterinario Veterinary use
	**Instalar até** Instalar hasta Install before		Ref.: 140214

Labtest