Creatinine

Jaffé. Colorimetric – kinetic

Quantitative determination of creatinine

CE IVD

PACKAGING

Ref.: 101-0281	Cont.: 2 x 100 mL
Ref.: 101-0453	Cont.: 8 x 100 mL
Ref.: 101-0623	Cont.: 2 x 1 L

Store at 2-8º C

CLINICAL SIGNIFICANCE

Creatinine is the result of the degradation of the creatine, component of muscles, it can be transformed into ATP, that is a source of high energy for the cells. The creatinine production depends on the modification of the muscular mass, and it varies little and the levels usually are very stable. Creatinine is excreted by the kidneys. With progressive renal insufficiency there is retention in blood of urea, creatinine and uric acid. Elevate creatinine level may be indicative of renal insufficiency[1,4,5].

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE OF THE METHOD

The assay is based on the reaction of creatinine with sodium picrate as described by Jaffé.

Creatinine reacts with alkaline picrate forming a red complex. The time interval chosen for measurements avoids interferences from other serum constituents.

The intensity of the color formed is proportional to the creatinine concentration in the sample[1].

REAGENTS

R 1 Picric Reagent	Picric acid	17.5 mmol/L
R 2 Alkaline Reagent	Sodium hydroxide	0.29 mol/L
CREATININE CAL	Creatinine aqueous primary standard 2 mg/dL	

Optional (not included in the kit)

Contro-N	Ref.: 101-0252	4 x 5 mL	Lyophilized human control serum
	Ref.: 101-0083	20 x 5 mL	
Contro-P	Ref.: 101-0253	4 x 5 mL	Lyophilized human control serum
	Ref.: 101-0084	20 x 5 mL	

PRECAUTIONS

R1(Picric acid)/ R2(NaOH) / WR: Corrosive (C):R35:Causes severe burns. S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S37/39: Wear suitable gloves and eye/face protection. S45: In case of accident or if you feel unwell, seek medical advices immediately.

PREPARATION

Working reagent (WR):

Mix equal volumes of R 1 Picric Reagent and R 2 Alkaline reagent.

The working reagent is stable for 15 days at 2 - 8º C or 7 days at room temperature (15 - 25º C).

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2 - 8º C, protected from light and contaminations prevented during their use.

Do not use reagents over the expiration date.

Signs of reagent deterioration:

- Presence of particles and turbidity.
- Blank absorbance (A) at 492 nm ≥ 1.80.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 492 nm (490-510).
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

SAMPLES

- Serum or heparinized plasma[1].
  Creatinine stability: 24 hours at 2 - 8º C.
- Urine (24h)[1]: Dilute sample 1/50 with distilled water. Mix. Multiply results by 50 (dilution factor);
  Creatinine stability: 7 days at 2 - 8º C.

PROCEDURE

Notes: CHRONOLAB SYSTEMS has instruction sheets for several automatic analyzers.

CREATININE CAL: Proceed carefully with this product because due its nature it can get contamined easily.

Calibration with the aqueous Standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.

Use clean disposable pipette tips for its dispensation.

1. Assay conditions:
   Wavelength: . . . . . 492 nm (490-510)
   Cuvette: . . . . . 1 cm. light path
   Temperature. . . . . 37º C / 15 - 25º C
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

	Blank	Standard	Sample
WR (mL)	1.0	1.0	1.0
Standard[(Note 1,2)] (µL)	--	100	--
Sample (µL)	--	--	100

4. Mix and start stopwatch.
5. Read the absorbance (A_1) after 30 seconds and after 90 seconds (A_2) of the sample addition.
6. Calculate: $\Delta A = A_2 - A_1$.

CALCULATIONS

$$\frac{\Delta A \text{ Sample} - \Delta A \text{Blank}}{\Delta A \text{ Standard} - \Delta A \text{Blank}} \times 2 \text{ (Standard conc.)} = \text{mg/dL of creatinine in the sample}$$

Conversion factor: mg/dL x 88.4 = µmol/L.

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures.

If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[1]

Serum or plasma:

Male	0.7 – 1.4 mg/dL	≅ 61.8 – 123.7 µmol/L
Female	0.6 – 1.1 mg/dL	≅ 53.0 – 97.2 µmol/L

Urine: 15-25 mg/Kg/24 h

Male	10 - 20 mg/Kg/24 h
Female	8 – 18 mg/Kg/24 h

These values are for orientation purpose; each laboratory should establish its own reference range.

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PERFORMANCE CHARACTERISTICS

Measuring range: From detection limit of 0.000 mg/dL to linearity limit of 35 mg/dL.
If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl (9 g/L) and multiply the result by 2.

Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (mg/dL)	0.92	3.43	0.96	3.50
SD	0.03	0.07	0.04	0.09
CV (%)	2.76	1.90	3.97	2.51

Sensitivity: 1 mg/dL = 0.0407 ΔA/min .

Accuracy: Results obtained using CHRONOLAB reagents (y) did not show systematic differences when compared with other commercial reagents (x).
The results obtained using 50 samples were the following:
Correlation coefficient (r): 0.99584
Regression equation: y= 0.953x + 0.075
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Hemoglobin (1 g/L), Bilirrubin (55 mg/dL), interfere[1].
A list of drugs and other interfering substances with creatinine determination has been reported[2,3].

BIBLIOGRAPHY

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