CREATININE
(FIXED RATE)

INTENDED USE

Vitro creatinine reagent is intended for the in vitro quantitative determination of creatinine in serum, plasma and urine on both automated and manual systems.

METHOD

Kinetic colorimetric method (Fixed rate) Jaffé reaction without deproteinization. .

CLINICAL SIGNIFICANCE

Serum creatinine is a waste product formed by the spontaneous dehydration of creatine. Most of the body creatine is found in muscle tissue where it is present as creatine phosphate and serves as a high-energy storage reservoir for conversion to adenosine triphosphate. The rate of creatinine formation is fairly constant with 1-2% of the body creatine being converted to creatinine every 24 hours. Serum creatinine and urea levels are elevated in patients with renal malfunction especially decreased glomerular filtration. In the early stages of kidney damage, the rise in the serum urea levels usually precedes the increase in serum creatinine. The advantage is offset by the fact that serum urea levels are affected by factors such as diet, degree of hydration and protein metabolism. Serum creatinine levels on the other hand tend to be constant and unaffected by factors affecting serum urea levels. Thus serum creatinine is a significantly more reliable renal function-screening test than serum urea. A considerably more sensitive test for measuring glomerular filtration is the creatinine clearance test. For this test precisely timed urine collection (usually 24 hours) and a blood sample are needed[1].

ASSAY PRINCIPLE

In 1886 Jaffe described a method for the measurement of creatinine in biological fluids[2]. This method involved precipitation of protein. Although several methods have been described since then, the original Jaffé technique is still the most widely used today. Vitro creatinine reagent is based on modified Jaffe reaction.

Creatinine in alkaline solution forms a yellow-red complex with alkaline picrate.

Creatinine + Picric acid $\xrightarrow{\text{Alkaline pH}}$ **Creatinine-Picric acid complex**

The rate of dye formation (color intensity) is directly proportional to the creatinine concentration in the specimen. It is determined by measuring the increase in absorbance at 480 - 520 nm.

EXPECTED VALUES

Serum or plasma[1]	
Males	0.9 - 1.5 mg/dl 80 – 133 µmol/l
Females	0.7 - 1.3 mg/dl 62 – 115 µmol/l
Urine[1]	
Males	14 - 26 mg/kg/day 0.124 –0.23 mmol/kg/day
Females	11 - 20 mg/kg/day 0.097–0.177 mmol/kg/day
Creatinine Clearance	
Males	90 – 139 ml/min
Females	80 – 125 ml/min

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes, the creatinine results should always be assessed in conjunction with the patient's medical history, clinical examination, and other findings.

REAGENTS

R_1	Creatinine standard	2.0	mg/dl
R_2	Picric acid	38	mmol/l
R_3	Sodium hydroxide	0.4	mol/l

- **Reagent Preparation & Stability**

All reagents are ready for use and stable up to the expiry date given on label when stored at 15 - 25^{0}C.

Working solution (R_2 + R_3):

According to requirements, prepare the working solution by mixing equal volumes of R_2 and R_3. The working solution is stable for 6 hours at 20 - 25 ^{0}C, when stored in a dark bottle.

SPECIMEN

- Serum, plasma, or urine.
- The only acceptable anticoagulants are heparin and EDTA.

Specimen Preparation & Stability

- **For serum or plasma specimen**

No special preparation of the patient is necessary. Creatinine remains stable in serum specimen for at least 7 days at 4^{0}C, and indefinitely when frozen [1, 3].

- **For urine specimen**

Creatinine in urine is stable for 2-3 days at room temperature and for at least 5 days refrigerated. Collect urine without additives. If urine must be collected with a preservative for other analytes, only thymol or toluene may be used. Urine specimens diluted 1:50 (1+49) with water prior to analysis[1, 3].

PROCEDURE

- **Manual Procedure**

Wavelength	480 - 520 nm
Cuvette	1 cm light path
Temperature	20 - 25 ^{0}C
Zero adjustment	against air or H_2O
Specimen	Serum, plasma or urine

Pipette into test tube or cuvette	
Working solution	1 ml
Standard or specimen	100 µl

Mix, after 30 sec. read initial absorbance (A_1). After exactly 2 min. later, read absorbance (A_2).

- **Automated Procedure**

User defined parameters for different auto analyzers are available upon request.

CALCULATION

Calculate the absorbance of standard and specimens by using the following formulae:

Absorbance of standard or specimen = (A_2 - A_1)

Then calculate the creatinine concentration using the following formulae:

Creatinine Concentration=

$$\frac{\text{Absorbance of Specimen}}{\text{Absorbance of Standard}} \times \text{Standard value}$$

For urine specimen the results must be multiplied by the dilution factor and 24 hours collections by the volume in liters.

- **Unit conversion**

mg/dl x 88.4= µmol/l

Creatinine Clearance

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EC REP **Medical Device Safety Services**
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Determine serum creatinine (mg/dl).
Determine urine creatinine (mg/dl).
Measure urine volume / 24 hours (ml).
Then calculate the creatinine clearance by using the following formulae:

$$\text{Creatinine clearance (ml/min)} = \frac{\text{Urine creatinine X Urine volume}}{\text{Serum creatinine X 1440}}$$

QUALITY CONTROL

It is recommended that controls (normal and abnormal) be included in:

- Each set of assays, or
- At least once a shift, or
- When a new bottle of reagent is used, or
- After preventive maintenance is performed or a clinical component is replaced.

Commercially available control material with established creatinine values may be routinely used for quality control.
Failure to obtain the proper range of values in the assay of control material may indicate:

- Reagent deterioration,
- Instrument malfunction, or
- Procedure errors.

The following corrective actions are recommended in such situations:

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
- If results are still out of control, contact Vitro Technical Services.

INTERFERING SUBSTANCES

- **Anticoagulants:**
  Heparin and EDTA are the only accepted anticoagulants.
- **Bilirubin:**
  Bilirubin levels higher than 5.0 mg/dl decrease the apparent creatinine concentration significantly.
- **Drugs:**
  Antibiotics containing cephalosporin lead to significant false-positive values. Young[4] in 1990 has published a comprehensive list of drugs and substances, which may interfere with this assay.
- **Haemoglobin:**
  No significant interference from haemoglobin up to a level of 1000 mg/dl.
- **Lipemia:**
  Intralipid levels higher than 250 mg/dl interfere with the creatinine test. Interference may be positive or negative.
- **Others:**
  No significant interference by acetone up to 50 mg/dl, acetoacetate up to 20 mmol/l.

WARNING & PRECAUTION

- Vitro creatinine reagent is for in vitro diagnostic use only. Normal precautions exercised in handling laboratory reagents should be followed.
- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- Valid results depend on an accurately calibrated instrument, timing, and temperature control.
- Don't use the reagent if it is turbid.
- Turbid or chylous specimens may produce erratic results. It is recommended that such specimens be centrifuged prior to testing.
- Urine specimen should be boiled briefly before testing.
- Don't pipette reagents by mouth. Wear protective clothing and gloves when handling the picric solution and working solution as both of these solutions stain clothing and skin. If spilled, flush with copious amounts of water.

PERFORMANCE CHARACTERISTICS

Imprecision

Reproducibility was determined using in an internal protocol. The following results were obtained.

	Within Run		Between Day	
Control	Level I	Level II	Level I	Level II
Number of samples	40	40	40	40
Mean (g/dl)	2.1	7.1	2.2	7.2
SD (g/dl)	0.05	0.09	0.09	0.18
CV (%)	2.1	1.2	4.1	2.3

Method Comparison

Comparison studies were carried out using a similar commercially available Creatinine reagent as a reference. Serum samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained.
Number of sample pairs 45
Range of sample results 0.68-13.20 mg/dL
Mean of reference method results 2.4 mg/dl
Mean of Creatinine results 2.3 mg/dl
Slope 0.95
Intercept 0.04 mg/dl
Correlation coefficient 0.998

Sensitivity

The sensitivity is defined as the change of analytical response (ΔA/min) per unit change in analyte concentration at a pathlength of 1 cm.

When run as recommended the sensitivity of this assay is 0.1 mg/dl (8.8 µmol/l).

LINEARITY

When run as recommended, the assay is linear up to 20 mg/dl (1.77 mmol/l).
If result exceeds 20 mg/dl (1.77 mmol/l), specimen should be diluted with 0.9% NaCl solution and reassayed. Multiply the result by the dilution factor.

BIBLIOGRAPHY

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2. **Jaffé, M (1986):** ueber den Niederschlag, welchen Pikrinsäure in normalem Harn erzeugt und über eine neue Reaktion des Kreatinins. Z Physiol Chem. 10:391-400.
3. **Di Giorgio, J (1974):** Nonprotein nitrogenous consistituents in: Henry RJ, Cannon DC, Winkelman JW, eds. Clinical Chemistry: Principles and Technics. 2nd ed. New York: Harper & Row; 503-557.
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SYMBOL DECLARATION

	Manufacturer
	Consult instructions for use
	Batch code (Lot #)
	Catalog number
	Temperature limitation
	In vitro diagnostic medical device
	Use by
	Caution. Consult instructions
	Keep away from light

ORDERING INFORMATION

REF	SIZE	REF	SIZE
11101	2 X 60 ml	11105	4 X 125 ml
11102	2 X 100 ml	11106	2 x 250 ml
11103	2 X 125 ml	11107	4 x 250 ml
11104	4 x 100 ml	11108	2 x 500 ml

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