**GENERALITIES** (1)

Interconversion of phosphocreatine and creatine is a particular feature of the metabolism processes of muscle contraction. Creatine and phosphocreatine partially convert to a waste product, creatinine. Thus, the amount of creatinine produced each day is related to the muscle mass (and body weight), age, sex, diet or exercise and does not greatly vary from day to day.

**PRINCIPLE** (4) (5)

Colorimetric reaction (Jaffe reaction) of creatinine with alkaline picrate measured kinetically at 490 nm (490-510), without any pre-treatment step. This reaction has been improved (specificity, speed and adaptability) by the development of an initial-rate method.

**REAGENTS COMPOSITION**

<table>
<thead>
<tr>
<th>REAGENT</th>
<th>COMPOSITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1 CREATININE</td>
<td>Reagent 1, Disodium Phosphate 6.4 mmol/L, Sodium hydroxide 150 mmol/L</td>
</tr>
<tr>
<td>R2 CREATININE</td>
<td>Reagent 2, Sodium dodecyl sulfate 0.75 mmol/L, Picric acid 4.0 mmol/L, pH 4.0</td>
</tr>
</tbody>
</table>

Attention: Met Corr.1: H290 - May be corrosive to metals, Skin Irrit.2: H315 - Causes skin irritation.

**SPECIMEN COLLECTION AND HANDLING** (2)

Serum or heparinised plasma.

Urine: Collect during precisely timed interval's (4, 12 or 24 h). Dilute 1+19 in demineralised water before determination.

Creatinine is stable for 24 h at 2-8°C.

**LIMITS** (1) (2) (3) (5)

Reading interval is the main determinant for the specificity of the Jaffe reaction; some interferents act quickly (acetoacetate) and others slowly (proteins). The majority of kinetic methods recommend a reading interval between 30 and 150 seconds. Some antibiotics interfere also with the determination of creatinine according to Jaffe method. For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

**MATERIAL REQUIRED BUT NOT PROVIDED**

1. Basic medical analysis laboratory equipment.
2. Spectrophotometer or Biochemistry Clinical Analyzer

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**SAFETY CAUTIONS**

- Refer to current Material Safety Data Sheet available on request or on www.biolabo.fr.
- Verify the integrity of the contents before use.
- Waste disposal: Respect legislation in force in the country.
- All specimens or reagents of biological origin should be handled as potentially infectious. Respect legislation in force in the country.
- Any serious incident that has occurred in connection with the device is notified to the manufacturer and the competent authority of the Member State in which the user and/or patient is based.

**REAGENTS PREPARATION**

Mix 1 volume of R1 and 1 volume of R2.

**STABILITY AND STORAGE**

Stored away from light, well caped in the original vial at 18-25°C, and used as described, reagents are stable:

- Unopened:
  - Until expiry date stated on the label of the kit.
  - Once opened:
    - Transfer requested quantity, well recap vials and store at 18-25°C
    - Separate reagents are stable at least 1 year.
  - Once reconstituted and free from contamination:
    - Reagent (R1+R2) is stable 30 days at 2-8°C
    - Discard reagent if cloudy or if its abs. is > 0.300 at 490 nm.
  - Don’t use working reagent after expiry date

**I INTENDED USE**

This reagent is designated for professional use in laboratory (manual or automated method).

It allows the quantification of creatinine in human serum and plasma or urines to screen its level.

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**REFERENCE INTERVALS (2)**

<table>
<thead>
<tr>
<th>Serum or plasma</th>
<th>[µmol/L]</th>
<th>mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>[80-115]</td>
<td>0.9 to 1.3</td>
</tr>
<tr>
<td>Female</td>
<td>[53-97]</td>
<td>0.6 to 1.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Urines</th>
<th>[µmol/kg/24h]</th>
<th>mg/kg/24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>[124-230]</td>
<td>14 to 26</td>
</tr>
<tr>
<td>Female</td>
<td>[97-177]</td>
<td>11 to 20</td>
</tr>
</tbody>
</table>

**PERFORMANCES**

On Kenza 240TX, 37°C, 505 nm (2 separate reagents) with seric specimens

Detection limit: 4.4 µmol/L (0.05 mg/dL)

Linearity Range: between 22 and 1328 µmol/L (15 mg/dL)

Precision:

<table>
<thead>
<tr>
<th>Within-run</th>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
<th>Between run</th>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 20</td>
<td></td>
<td></td>
<td></td>
<td>N = 20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (µmol/L)</td>
<td>58.4</td>
<td>141.6</td>
<td>506.9</td>
<td>Mean (µmol/L)</td>
<td>58.4</td>
<td>145.1</td>
<td>514.2</td>
</tr>
<tr>
<td>S.D. µmol/L</td>
<td>1.06</td>
<td>1.86</td>
<td>8.14</td>
<td>S.D. µmol/L</td>
<td>2.3</td>
<td>5.13</td>
<td>11.6</td>
</tr>
<tr>
<td>C.V. %</td>
<td>1.8</td>
<td>1.3</td>
<td>1.6</td>
<td>C.V. %</td>
<td>4.0</td>
<td>3.5</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Analytical sensitivity: approx. 0.018 abs/120 sec for 1 mg/dL (88,5 µmol/L)

Comparison studies with commercially available reagent:

Realised on human specimens (n=123) between 0.41 and 13.6 mg/dL

Comparison studies with commercially available reagent:

GLomerular filtration rate (mL per minute)

Adult < 40 years: 120 (100 – 140)

Adult > 40 years: Physiologically decreased approx. 1% every year.

Each laboratory should establish its own normal ranges for the population that it serves.

**QUALITY CONTROL**

- REF 95015 Multicallibrator: value traceable to SRM967 for quantitative determination in serum/plasma.
- REF 80107 Standard (vial R3):
  - Value traceable to SRM914 for quantitative determination in urines.
  - Value traceable to SRM967 for quantitative determination in urines/serum.

According to ANSM: 1 zero-point, 1 intermediate level and 1 high level have been used to determine these values.

The calibration frequency depends on proper instrument functions and on preservation of the reagent.

**PROCEDURE**

Manual method

Let stand reagent and specimens at room temperature.

<table>
<thead>
<tr>
<th>Working Reagent (R1+R2)</th>
<th>Specimen (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000 µL</td>
<td>100 µL</td>
</tr>
</tbody>
</table>

Mix well. Perform kinetic tests at 37°C (verify constant temperature). After 30 seconds read absorbance A1 and exactly 120 sec after read absorbance A2 at 490 nm (490-510) against distilled water. Test tube by tube with water (Blank), calibrator, controls and then assaies as specimen.

1- Performances with manual procedure and with urines should be validated by user.
2- Kenza applications and other applications proposal are available on request.
3- Calibration: use calibrator or aqueous standard as indicated in §Calibration
4- Specimen: serum, plasma or pre-diluted urines (1+19) in demineralised water before measurement.

**CALCULATION (6)**

**Serum or plasma**

\[
\text{Result} = \frac{(A2 - A1) \text{ Assay} - (A2 - A1) \text{ Blank}}{x \text{ Standard Concentration}}
\]

**Urine diluted with 1+19:**

Multiply the above result by dilution factor 20

GFR (by creatinine clearance determination):

\[
\text{GFR} = \frac{\text{UCr} \times V \times 1.73}{\text{SCr} \times \text{BSA}}
\]

Where:

- UCr = Urine Creatinine in mg/dL or µmol/L
- SCr = Serum Creatinine in mg/dL or µmol/L
- V = Urine volume excreted in mL/min (24 h urine volume/1440)
- BSA = Body Surface Area in m²

**OR**

Using only serum creatinine (by Cockcroft and Gault formula):

\[
\text{Creatinine Clearance} = \frac{140 - \text{age in years} + 2.12 \times \text{weight in Kg}}{\text{Serum Creatinine (µmol/L) \times BSA (m²)}}
\]

Where:

- K = 1.00 for men or K = 0.85 for women

**REFERENCES**

3. YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p.3-190 to 3-211
6. SRM: Standard Reference Material®