**CLINICAL SIGNIFICANCE**

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. Because creatinine is endogenously produced and released into body fluids at a constant rate and its plasma levels are maintained within narrow limits, its clearance can be measured as an indicator of glomerular filtration rate (GFR).

**PRINCIPLE**

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>Vial R1</th>
<th>BASE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xi: IRRITANT , R36/38: Irritating to eyes and skin. S36/37/39: Wear suitable protective clothing, gloves and eyes/face protection</td>
<td></td>
</tr>
<tr>
<td>Disodium Phosphate 6.4 mmol/L</td>
<td></td>
</tr>
<tr>
<td>Sodium hydroxide 150 mmol/L</td>
<td></td>
</tr>
<tr>
<td>pH 4.0</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vial R2</th>
<th>DYE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium dodecyl sulfate 0.75 mmol/L</td>
<td></td>
</tr>
<tr>
<td>Picric acid 4.0 mmol/L</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vial R3</th>
<th>STANDARD</th>
</tr>
</thead>
<tbody>
<tr>
<td>177 µmol/L (2 mg/dL)</td>
<td></td>
</tr>
</tbody>
</table>

**SAFETY CAUTIONS**

BIOLABO reagents are designated for professional, in vitro diagnostic use.

- Verify the integrity of the contents before use.
- Use adequate protections (overall, gloves, glasses).
- Do not pipette by mouth.
- In case of contact with skin or eyes, thoroughly wash affected areas with plenty of water and seek medical advice.
- Material Safety Data Sheet is available upon request.
- Waste disposal: Respect legislation in force in the country.

**MATERIAL REQUIRED BUT NOT PROVIDED**

1. Basic medical analysis laboratory equipment.
2. Normal and pathological control sera.

**REAGENTS PREPARATION**

Mix vial R1 and vial R2 contents (1 volume/1 volume). A graduated test-tube may be used.

**TECHNICAL SUPPORT AND ORDERS**

Tel : (33) 03 23 25 15 50
Fax: (33) 03 23 25 265

**MANUFACTURER:**
BIOLABO SAS, Les Hautes Rives 02160, Maizy, France

**REALTIONAL USE**

**STABILITY AND STORAGE**

Store at 18-25°C, well cap in the original vial and away from light.

- Standard (vial R3): Transfer the requested quantity, recap and store at 18-25°C.
- Reagents are stable until expiry date stated on the label of the kit when free from contamination, stored and used as described in the insert.
- Once reconstituted, working reagent is stable for 30 days at 2-8°C when free from contamination.

**INTERFERENCES**

(1) (2) (3) (5)

<table>
<thead>
<tr>
<th>Procedure n°1:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (µmol/L) In specimen</td>
</tr>
<tr>
<td>249 µmol/L Glucose</td>
</tr>
<tr>
<td>115 µmol/L Proteins</td>
</tr>
<tr>
<td>99 µmol/L Ascorbic acid</td>
</tr>
<tr>
<td>106 µmol/L Bilirubin</td>
</tr>
<tr>
<td>96 µmol/L Haemoglobin</td>
</tr>
<tr>
<td>105 µmol/L Lipemia</td>
</tr>
</tbody>
</table>

| Procedure n°2: No interference of Bilirubin |
| 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. |

**CALIBRATION**

- Kit Standard (vial R3) or BIOLABO Multicalibrator, 95015 traceable to SRM 909b (ID-MS) or SRM914a/SRM967a validated according to the recommendations of AFSSAPS (1 zero point, 1 point within normal level, 1 point within high level).
- Or any calibrator traceable to a reference method or material.

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

It is recommended to calibrate in the following cases:
1. When changing batch of reagent.
2. After maintenance operations on the instrument.
3. When control values obtained are out of range, even after using a new vial of fresh serum.

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Fax: (33) 03 23 25 265
QUALITY CONTROL
- BIOLABO EXATROL-N Level I (REF 95010).
- BIOLABO EXATROL-P Level II (REF 95011).
- Assayed control sera referring to the same method.
- AFSSAPS recommends to use low control, subnormal control and pathological control.
- External quality control program.

It is recommended to control in the following cases:
- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If control is out of range, apply following actions:
1. Repeat the test with the same control.
2. If control is still out of range, prepare a fresh control serum and repeat the test.
3. If control is still out of range, use a new vial of calibrator or a fresh calibrator and repeat the test.
4. If control is still out of range, calibrate with a new vial of reagent.
5. If control is still out of range, please contact BIOLABO technical support or your local Agent.

EXPECTED VALUES (2)

**Serum or plasma**

<table>
<thead>
<tr>
<th>Creatinine</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>Creatinine</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>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>[97-177]</td>
<td>11 to 20</td>
<td></td>
</tr>
</tbody>
</table>

**GFR (Glomerular filtration rate) mg/min**

- 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.

PERFORMANCES (PROCEDURE N°1)

<table>
<thead>
<tr>
<th>Low level</th>
<th>Mean µmol/L</th>
<th>S.D. µmol/L</th>
<th>C.V. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>54.4</td>
<td>2.12</td>
<td>3.9</td>
</tr>
<tr>
<td>Female</td>
<td>117</td>
<td>1.41</td>
<td>1.2</td>
</tr>
<tr>
<td>High level</td>
<td>323</td>
<td>2.65</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Detection limit: approximately 18 µmol/L at 37°C.

Sensitivity for 1 mg/dL: approximately 18 mAbs/min at 37°C.

**Comparison study with commercially available reagent** (Jaffé Kinetic Method):

- 60 sera within 44.2 and 884 µmol/L have been evaluated with both reagents:
  \[ y = 1.06 \times x - 5.4 \quad r = 0.9981 \]

<table>
<thead>
<tr>
<th>Units (µmol/L)</th>
<th>Y calculated value</th>
<th>Observed Inaccuracy</th>
<th>Acceptable Inaccuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>50.4</td>
<td>48.7</td>
<td>-1.7</td>
<td>7.97</td>
</tr>
<tr>
<td>139.8</td>
<td>143.4</td>
<td>3.8</td>
<td>14.2</td>
</tr>
<tr>
<td>593</td>
<td>624.8</td>
<td>31.8</td>
<td>47.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Units (µmol/L)</th>
<th>Reference</th>
<th>BIOLABO</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean n=60</td>
<td>104.8</td>
<td>106.3</td>
<td>+1.5</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>79.1</td>
<td>83.9</td>
<td>+4.8</td>
</tr>
</tbody>
</table>

LINEARITY
The assay is linear up to 1327 µmol/L (15 mg/dL). Above, dilute the specimen (1/4) with saline solution and reassy taking into account the dilution factor. Linearity limit depends on specimen/reagent ratio.

MANUAL PROCEDURE
Let stand reagents and specimens at temperature of measurement. Perform all the assays at constant temperature (see note 4).

**Procedure n°1:** For non icteric specimen using "Working reagent"

<table>
<thead>
<tr>
<th>Pipette in a 1 cm path cuvette:</th>
<th>Blank (optional)</th>
<th>Standard</th>
<th>Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working reagent (R1 + R2)</td>
<td>1 mL</td>
<td>1 mL</td>
<td>1 mL</td>
</tr>
<tr>
<td>Demineralised water</td>
<td>100 µL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>100 µL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specimen (Note 1)</td>
<td>100 µL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mix well. After 30 seconds, record absorbance A1 at 490 nm (490-510) against reagent blank or distilled water. Exactly 2 minutes after the first reading, record absorbance A2.

**Procedure n°2:** For icteric specimen using "Bi-reagent"

<table>
<thead>
<tr>
<th>Pipette in a 1 cm path cuvette:</th>
<th>Blank (optional)</th>
<th>Standard</th>
<th>Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent R1</td>
<td>0.5 mL</td>
<td>0.5 mL</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>Demineralised water</td>
<td>100 µL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>100 µL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specimen (Note 1)</td>
<td>100 µL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Incuclate for 5 minutes at constant temperature, then add:

- Reagent R2: 0.5 mL
- Blank: 0.5 mL
- Standard: 0.5 mL

Mix well. After 30 seconds, record absorbance A1 at 490 nm (490-510) against reagent blank or distilled water. Exactly 2 minutes after the first reading, record absorbance A2.

Notes:
1. Specimen: serum, plasma, or diluted urines 1+19 in distilled water.
2. Reading interval is the main determinant for the specificity of the Jaffé reaction; some interferents act quickly (acetocetate) and others slowly (proteins). The majority of kinetic methods recommend a reading interval between 30 and 150 seconds.
3. Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support.
4. Perform this test at 37°C to optimise the sensitivity.

**CALCULATION (6)**
Calculate the result as follows:

**Using 24 h urine and serum creatinine**

- Corrected Creatinine Clearance (mL/min) = \( \frac{UCr \times V \times 1.73}{SCr \times BSA} \)
- 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)**

- Creatinine Clearance = (140 – age in years) x 2.12 x weight in Kg x K / SCr x BSA

- 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®