**PROTOCOL**

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

**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. 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.

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

**REAGENTS**

1. **Reagent 1 (Picric Reagent)**
   - Picric acid 17.5 mmol/L
   - Sodium hydroxide 0.29 mol/L
2. **Reagent 2 (Alkaline Reagent)**
   - Standard concentration see on the vial label

**PRECAUTIONS**

Sodium hydroxide: Irritant (Xi): R36/38: Irritating to eyes and skin. S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S37/39: Wear suitable gloves and eyewear 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 10 days at +15 to +25°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 to +8°C, protected from light and contaminations prevented during their use.

Do not use reagents over the expiration date.

**SAMPLE**

- Serum or plasma
- Urine: Dilute sample 1/50 with distilled water. Mix. Multiply results by 50 (dilution factor)

**PROCEDURE**

1. **Assay conditions:**
   - Wavelength: 492 nm (490-510)
   - Temperature: 37°C / 15-25°C
   - Blank absorbance (A) at 492 nm
   - Matched cuvettes 1.0 cm light path.
   - Hemoglobin (1 g/L), Bilirubin (55 mg/dL), interfere. A list of drugs and other interfering substances with creatinine determination has been reported by Young et. al.
   - 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.
   - Calibration with the aqueous Standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
   - Sensitivity: 1 mg/dL = AA 0.03 A/min . mg/dL
   - Accuracy: Results obtained using CHRONOLAB reagents (y) did not show systematic differences when compared with other commercial reagents (x).
   - Stability of the sample: Creatinine is stable for 7 days at +2 to +8°C.

**PERFORMANCE CHARACTERISTICS**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
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<tr>
<td></td>
<td>--</td>
<td>100</td>
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<td>100</td>
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</tbody>
</table>

4. Mix and start stopwatch.
5. Read the absorbance (A1) after 30 seconds and after 90 seconds (A2) of the sample addition.
6. Calculate: \( \Delta A = A2 - A1 \).

**CALCULATIONS**

\[ \Delta A \times (\text{Standard conc.}) = \text{mg/dL of creatinine in the sample} \]

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

**QUALITY CONTROL**

Control sera are recommended to monitor the performance of assay procedures with Contro N and Contro P.

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**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Serum or Plasma</th>
<th>Male</th>
<th>Female</th>
<th>Urine</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0.7 – 1.4 mg/dL</td>
<td>61.8 – 123.7 μmol/L</td>
<td>53.0 – 97.2 μmol/L</td>
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</tr>
<tr>
<td></td>
<td>0.6 – 1.1 mg/dL</td>
<td>52.0 – 100.0 μmol/L</td>
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</tr>
</tbody>
</table>

**INTERFERENCES**

Hemoglobin (1 g/L), Bilirubin (55 mg/dL), interfere.

A list of drugs and other interfering substances with creatinine determination has been reported by Young et. al.

**NOTES**

1. Calibration with the aqueous Standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
2. Use clean disposable pipette tips for its dispensation.
3. CHRONOLAB has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

**REFERENCES**