Quantitative determination of Creatinine

Only for in vitro use in clinical laboratory (IVD)

**TEST SUMMARY**
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.

**REAGENTS COMPOSITION**

<table>
<thead>
<tr>
<th>R1 (Picric Reagent)</th>
<th>R2 (Alkaline Reagent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Picric Acid</td>
<td>Sodium hydroxide</td>
</tr>
<tr>
<td>17.5 mmol/L</td>
<td>0.29 mmol/L</td>
</tr>
</tbody>
</table>

Creatinine Cal Creatinine aqueous primary calibrator 2 mg/dL.

**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 advice immediately.

**REAGENT PREPARATION AND STABILITY**
Working reagent (WR): Mix equal volumes of R.1 (Picric Reagent) and R.2 (Alkaline Reagent). The working reagent (WR) is stable for 15 days at 2-8°C or 7 days at room temperature (15-25°C). All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use. Do not use reagents over the expiration date.

Creatinine Cal Proceed carefully with this product because due its nature it can get contaminated easily.

**Signs of Reagent deterioration:**
- Presence of particles and turbidity.
- Blank absorbance (A) at 492 nm. ≥ 1.80

All the reagents of the kit are stable up to the end of the indicated month and year of expiry. Store tightly closed at 2-8°C. Do not use reagents over the expiration date.

**SPECIMEN**
- Serum or heparinized plasma
- Creatinine stability: 24 hours at 2-8°C
- Urine: Dilute sample 1:50 with distilled water. Mix. Multiply results by 50 (dilution factor).
- Creatinine stability: 7 days at 2-8°C

**MATERIAL REQUIRED BUT NOT PROVIDED**
- Spectrophotometer or colorimeter measuring at 482nm.
- Matched cuvettes 1.0 cm. light path.

**TEST PROCEDURE**

1. **Assay Conditions**
   - Cuvette: 1.0 cm light path.
   - Temperature: 37°C. 15-25°C.
   - Adjust the instrument to zero with distilled water.

2. Mix and start stopwatch.
3. Read the absorbance (A) after 30 seconds and after 90 seconds [(A) of the sample addition.

**CALCULATIONS**
Creatinine (mg/dL) = (ΔA)Sample / (ΔA)Calibrator x 2 (Calibrator conc.)

Conversion Factor: mg/dL x 88.4 = µmol/L

**QUALITY CONTROL**
Control sera are recommended to monitor the performance of the procedure. LABTROL H Normal Ref. 30950 and LABTROL H Pathological Ref. 30955. If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

**REFERENCE VALUES**

<table>
<thead>
<tr>
<th>Serum or plasma:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male 0.7 – 1.4 mg/dL = 61.8 – 123.7 µmol/L</td>
</tr>
<tr>
<td>Female 0.6 – 1.1 mg/dL = 53.0 – 97.2 µmol/L</td>
</tr>
</tbody>
</table>

Urine: 15-25 mg/Kg/24h

<table>
<thead>
<tr>
<th>Male</th>
<th>10 - 20 mg/Kg/24 h = 88– 177 µmol/Kg/24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>8 - 18 mg/Kg/24 h = 71 – 177 µmol/Kg/24 h</td>
</tr>
</tbody>
</table>

(These values are for orientation purpose).

It is suggested that each laboratory establish its own reference range.

**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.

**INTERFERING SUBSTANCES**

- 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.

**BIBLIOGRAPHY**