**INTENDED USE**

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

**BACKGROUND**

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 needed6.

**ASSAY PRINCIPLE**

In 1886 Jaffé described a method for the measurement of creatinine in biological fluids7. Although several methods have been described since then, the original Jaffé technique is still the most widely used today.

1. After deproteinization creatinine in alkaline solution, forms a yellow-red complex with picrate.

The intensity of the color produced is directly proportional to creatinine concentration. It is determined by measuring the increase in absorbance at 500 – 550 nm.

**EXPECTED VALUES**

<table>
<thead>
<tr>
<th>Serum or plasma1</th>
<th>Males</th>
<th>0.9 - 1.5 mg/dl</th>
<th>80 - 133 μmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>0.7 - 1.3 mg/dl</td>
<td>62 - 115 μmol/l</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Urine1</th>
<th>Males</th>
<th>14 - 26 mg/kg/day</th>
<th>0.124 - 0.23 mmol/kg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>11 - 20 mg/kg/day</td>
<td>0.097 - 0.177 mmol/kg/day</td>
<td></td>
</tr>
</tbody>
</table>

**Creatinine Clearance**

<table>
<thead>
<tr>
<th>Males</th>
<th>90 - 139 ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>80 - 125 ml/min</td>
</tr>
</tbody>
</table>

**METHOD**

Colorimetric method Jaffé reaction with deproteinization.

**SPECIMEN**

- Serum, plasma, or urine.
- The only acceptable anticoagulant is heparin.

**REAGENTS**

- **R₁**: Creatinine standard 2.0 mg/dl
- **R₂**: Picric acid 38 mmol/l
- **R₃**: Sodium hydroxide 1.2 mol/l

Additional reagent required but not provided:
- Trichloroacetic acid 1.2 mol/l

Please use Vitro Scient TCA.

**Reagent Preparation & Stability**

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

**Working solution (R₂ + R₃):**

According to requirements, prepare the working solution by mixing equal volumes of R₂ and R₃. The working solution is stable for 6 hours at 20-25°C, when stored in a dark bottle.

**Calculation**

Calculate the creatinine concentration by using the following formula:

**Creatinine Concentration:**

\[
\text{Creatinine Concentration} = \frac{X \cdot \text{Standard Absorbance}}{\text{Absorbance of Standard}}
\]

**Absorbance of Standard**

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

**Unit Conversion**

\[
\text{mg/dl} \times 88.4 = \mu\text{mol/l}
\]

**Creatinine Clearance**

Males | 1 ml |
--- | --- |
Females | 1 ml |

Mix well, centrifuge at 2500 rpm for 10 min. Collect the supernatant (protein free filtrate) (PFF). The PFF can be stored up to 7 days at 4°C.

**Calculation**

Calculate the creatinine concentration by using the following formula:

**Creatinine Concentration**

\[
\text{Creatinine Concentration} = \frac{X \cdot \text{Standard Absorbance}}{\text{Absorbance of Standard}}
\]

**Absorbance of Standard**

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

\[
\text{Creatinine clearance (ml/min)} = \frac{\text{Urine creatinine} \times \text{Urine volume}}{\text{Serum creatinine} \times 1.440}
\]

**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:** The only accepted anticoagulant is heparin.
- **Bilirubin:** Bilirubin levels higher than 25 mg/dl decrease the apparent creatinine concentration significantly.
- **Drugs:** Antibiotics containing cephalosporin lead to significant false-positive values.
- **Haemoglobin:** No significant interference from haemoglobin up to a level of 1000 mg/dl.
- **Lipemia:** Intralipid levels higher than 500 mg/dl decrease the apparent creatinine concentration significantly.
- **Others:** No significant interference by acetone up to 50 mg/dl, acetocetate 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 an internal protocol. The following results were obtained:

<table>
<thead>
<tr>
<th></th>
<th>Within Run</th>
<th>Between Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Level I</td>
<td>Level II</td>
</tr>
<tr>
<td>Mean (g/dl)</td>
<td>2.1</td>
<td>7.1</td>
</tr>
<tr>
<td>SD (g/dl)</td>
<td>0.05</td>
<td>0.09</td>
</tr>
<tr>
<td>CV (%)</td>
<td>2.1</td>
<td>1.2</td>
</tr>
</tbody>
</table>

**Sensitivity**

The sensitivity is defined as the change of analytical response per unit change in analyte concentration at a path length of 1 cm.

When run as recommended, the sensitivity of this assay is 0.1 mg/dl (0.88 mmol/l).

If result exceeds 12 mg/dl (1.062 mmol/l), specimen should be diluted with 0.9% NaCl solution and reassayed. Multiply the result by the dilution factor.

**BIBLIOGRAPHY**


**ORDERING INFORMATION**

<table>
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<tr>
<th>REF</th>
<th>SIZE</th>
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<tbody>
<tr>
<td>1121</td>
<td>2 x 60 ml</td>
</tr>
<tr>
<td>1122</td>
<td>2 x 100 ml</td>
</tr>
<tr>
<td>1123</td>
<td>4 x 100 ml</td>
</tr>
</tbody>
</table>

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