**INTENDED USE**

Vitro creatinine reagent is intended for the in vitro quantitative determination of creatinine in serum, plasma and urine on both automated and 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 needed.

ASSAY PRINCIPLE

In 1886 Jaffé described a method for the measurement of creatinine in biological fluids. This method involved precipitation of protein. Although several methods have been described since then, the original Jaffé technique is still the most widely used today. Vitro creatinine reagent is based on modified Jaffé reaction.

Creatinine in alkaline solution forms a yellow-red complex with alkaline picrate.

\[
\text{Creatinine} + \text{Picric acid} \rightarrow \text{Alkaline Creatinine-Picrate complex}
\]

The rate of dye formation (color intensity) is directly proportional to the creatinine concentration in the specimen. It is determined by measuring the increase in absorbance at 480 - 520 nm.

**EXPECTED VALUES**

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

<table>
<thead>
<tr>
<th>Urine</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine Clearance</td>
<td>14 - 26 mg/kg/day</td>
<td>11 - 20 mg/kg/day</td>
</tr>
<tr>
<td>Concentration</td>
<td>0.124 - 0.23 mmol/kg/day</td>
<td>0.097 - 0.177 mmol/kg/day</td>
</tr>
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Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes, the creatinine results should always be assessed in conjunction with the patient’s medical history, clinical examination, and other findings.

**REAGENTS**

| R1 | Creatinine standard | 2.0 mg/dl |
| R2 | Picric acid | 38 mmol/l |
| R3 | Sodium hydroxide | 0.4 mol/l |

- **Reagent Preparation & Stability**

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

  **Working solution (R1 + R2):**

  According to requirements, prepare the working solution by mixing equal volumes of R1 and R2. The working solution is stable for 6 hours at 20 - 25°C, when stored in a dark bottle.

- **Specimen Preparation & Stability**

  - Serum, plasma, or urine.
  - The only acceptable anticoagulants are heparin and EDTA.

**PROCEDURE**

- **Manual Procedure**

  1. Pipette into test tube or cuvette
  2. Working solution 1 ml
  3. Standard or specimen 100 μl
  4. Mix, after 30 sec. read initial absorbance (A1). After exactly 2 min. later, read absorbance (A2).

- **Automated Procedure**

  User defined parameters for different auto analyzers are available upon request.

**CALCULATION**

Calculate the absorbance of standard and specimens by using the following formulae:

\[
\text{Absorbance of standard or specimen} = (A_2 - A_1)
\]

Then calculate the creatinine concentration using the following formulae:

\[
\text{Creatinine Concentration} = \frac{\text{Absorbance of Specimen}}{\text{Absorbance of Standard}} \times \text{Standard Creatinine Concentration}
\]

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

- **Unit conversion**

  mg/dl x 180/44 = μmol/l

**Creatinine Clearance**

<table>
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<td>62 – 80</td>
<td>1.3 mg/dl</td>
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<tr>
<td>0.7 – 1.0</td>
<td>0.23 mmol/kg/day</td>
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</table>

**Calculation**

1. Creatinine in urine is stable for 2-3 days at room temperature and for at least 5 days refrigerated. Collect urine without additives. If urine must be collected with a preservative for other analytes, only thymol or toluene may be used. Urine specimens diluted 1:50 (1+49) with water prior to analysis.

   - **REAGENTS**
     - R1: Creatinine standard 2.0 mg/dl
     - R2: Picric acid 38 mmol/l
     - R3: Sodium hydroxide 0.4 mol/l

   - **Specimen**
     - Serum, plasma, or urine
     - The only acceptable anticoagulants are heparin and EDTA.

   **Calculation**

   Calculate the absorbance of standard and specimens by using the following formulae:

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**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 formulae:

\[
\text{Creatinine clearance (ml/min)} = \frac{\text{Urine creatinine} \times \text{Urine volume}}{\text{Serum creatinine} \times 1440}
\]

**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 preventative 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:**
  - Heparin and EDTA are the only accepted anticoagulants.

- **Bilirubin:**
  - Bilirubin levels higher than 5.0 mg/dl decrease the apparent creatinine concentration significantly.

- **Drugs:**
  - Antibiotics containing cephalosporin lead to significant false-positive values. Young* in 1990 has published a comprehensive list of drugs and substances, which may interfere with this assay.
  - Haemoglobin:
  - No significant interference from haemoglobin up to a level of 1000 mg/dl.
  - Lipemia:
  - Intralipid levels higher than 250 mg/dl interfere with the creatinine test. Interference may be positive or negative.
  - Others:
  - No significant interference by acetone up to 50 mg/dl, acetacetate 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>Control</th>
<th>Within Run</th>
<th>Between Day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Level I</td>
<td>Level II</td>
</tr>
<tr>
<td>Number of samples</td>
<td>40</td>
<td>40</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>

**Method Comparison**

Comparison studies were carried out using a similar commercially available Creatinine reagent as a reference. Serum samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained.

- **Number of sample pairs**: 45
- **Range of sample results**: 0.68-13.20 mg/dl
- **Mean of reference method results**: 2.4 mg/dl
- **Mean of Creatinine results**: 2.3 mg/dl
- **Slope**: 0.95
- **Intercept**: 0.04 mg/dl
- **Correlation coefficient**: 0.998

**Sensitivity**

The sensitivity is defined as the change of analytical response (ΔA/min) per unit change in analyte concentration at a pathlength of 1 cm.

When run as recommended the sensitivity of this assay is 0.1 mg/dl (8.8 μmol/l).

**LINEARITY**

When run as recommended, the assay is linear up to 20 mg/dl (1.77 mmol/l).

If result exceeds 20 mg/dl (1.77 mmol/l), specimen should be diluted with 0.9% NaCl solution and reassayed. Multiply the result by the dilution factor.

**BIBLIOGRAPHY**


**SYMBOL DECLARATION**

**MANUFACTURER DECLARATION**

- Consult instructions for use
- Batch code (Lot #)
- Catalog number
- Temperature limitation
- In vitro diagnostic medical device
- Use by
- Caution. Consult instructions
- Keep away from light

**ORDERING INFORMATION**

<table>
<thead>
<tr>
<th>REF</th>
<th>SIZE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1111</td>
<td>2 x 60 ml</td>
</tr>
<tr>
<td>1112</td>
<td>2 x 100 ml</td>
</tr>
<tr>
<td>1113</td>
<td>4 x 100 ml</td>
</tr>
</tbody>
</table>

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