Creatinine – colorimetric

Intended Use
Spectrum Diagnostics creatinine reagent is intended for the in-vitro quantitative, diagnostic determination of creatinine in human serum or urine on manual system.

Background
Creatine is synthesized in kidney, liver and pancreas. It is transported in blood to other organs such as muscle and brain where it is phosphorylated to phosphocreatine. Some free creatine in muscle is converted to creatine daily, and the amount of creatinine produced is proportional to muscle mass. In the absence of renal disease, excretion rate of creatinine in an individual is relatively constant. Therefore measurement of creatinine clearance is useful in detecting renal disease and estimating the extent of impairment of renal function. Both serum creatinine and urea levels are elevated in patients with renal malfunction, especially decreased glomerular filtration. In the early stage of kidney damage, increase in serum urea level usually precedes the increase in serum creatinine. However, serum urea levels may be affected by dehydration, diet and protein metabolism. On the other hand serum creatinine levels tend to be constant and unaffected by such factors. Thus serum creatinine is a significantly more reliable renal function screening test than serum urea.

Method
Colorimetric method with deproteinization.

Assay Principle
Creatinine reacts with picric acid in alkaline solution to form a coloured complex.

Creatinine + picrate → yellow-red complex

Reagents
Standard creatinine (ST)
2 mg/dL 177 mmol/L

Reagent 1 (R1)
Picric acid 38 mmol/L
Reagent 1 contains a low concentration of picric acid, a chemical which, in its dry form, is flammable and potentially explosive. For this reason, it is recommended that drains be well flushed with water when discarding the reagent, spills be cleaned up at once, and dried material not be allowed to build up around the reagent bottle opening.

Irritant (Xi) material not be allowed to build up around the reagent bottle opening when discarding the reagent, spills be cleaned up at once, and dried

Risk of serious damage to eyes.

Avoid contact with skin and eyes.

Reagent 2 (R2)
Sodium hydroxide 1.6 mol/L
Reagent 2 contains caustic material.

Corrosive (C)

Risk of serious damage to eyes.

Avoid contact with skin and eyes.

For further information, refer to the Creatinine colorimetric reagent material safety data sheet.

Additional Reagent
Trichloroacetic acid 1.2 mol/L.

Reagent Preparation
Prepare working solution as following:
Combine one volume of R1 with one volume of R2, e.g. 1.0 ml R1 + 1.0 ml R2

Precautions and Warnings
Do not ingest or inhale. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.

Reagent Storage and Stability
All reagents are stable until expiration date stated on label when stored at 15 - 25 °C. Working solution is stable for 5 hours at 15 – 25 °C away from light.

Deterioration
The creatinine reagents are not suitable for use if combined reagents have an absorbance greater than 0.6 at 492 nm measured in a 1-cm lightpath or if the reagents develop a hazy appearance.

Specimen Collection and Preservation
Serum or plasma
Both are suitable for analysis. The only acceptable anticoagulants are heparin and EDTA. Specimen should be promptly separated from cells after blood collection. The biological half-life of creatinine in blood is few minutes.

Stability: 7 days 2 - 8 °C ; > 1 year at – 20 °C

Urine
Thymol or tolune may be used for urine preservation. To determine creatinine concentration in urine, dilute 1 part sample with 49 parts isotonic saline prior to assay. Multiply result by 50 to compensate for dilution.

Stability: 2 days at 15 - 25 °C ; 6 days at 2 - 8 °C
6 months at -20 °C away from light

System Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength</td>
<td>546 nm (500 - 550 nm)</td>
</tr>
<tr>
<td>Optical path</td>
<td>1 cm</td>
</tr>
<tr>
<td>Assay type</td>
<td>End-point</td>
</tr>
<tr>
<td>Direction</td>
<td>Increase</td>
</tr>
<tr>
<td>Sample : Reagent Ratio</td>
<td>1 : 1</td>
</tr>
<tr>
<td>e.g.: Reagent volume</td>
<td>1 ml</td>
</tr>
<tr>
<td>Sample volume</td>
<td>1 ml</td>
</tr>
<tr>
<td>Temperature</td>
<td>25 °C</td>
</tr>
<tr>
<td>Zero adjustment</td>
<td>Against Air</td>
</tr>
<tr>
<td>Reagent Blank Limits</td>
<td>Low 0.30 AU</td>
</tr>
<tr>
<td></td>
<td>High 0.70 AU</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.4 mg/dL (0.035 mmol/L)</td>
</tr>
<tr>
<td>Linearity</td>
<td>15 mg/dL (1.32 mmol/L)</td>
</tr>
</tbody>
</table>

Deproteinization Procedure
Pipe into centrifuge tubes
Trichloroacetic acid (TCA) 1.0 ml
Serum or heparinized plasma 1.0 ml
(TCA reagent is available upon request)
Mix well using a glass rod to disperse the precipitate. Centrifuge at 3000 rpm for 10 minutes, then pour off the supernatant into a clean tube.
Stability: the supernatant is stable for 7 days at 2 - 4 °C.

SYMBOLS IN PRODUCT LABELLING

- EC REP: Authorised Representative
- Use by/Expiration Date
- VD: For in-vitro diagnostic use
- Batch Code/Lot number
- LD: Catalogue Number
- Manufactured by
- S: Consult instructions for use
- (Xi): Irritant
- (C): Corrosive

Stability:
2 days at 15 - 25 °C ; 6 days at 2 - 8 °C
6 months at -20 °C away from light

Procedure
Pipette into test tubes

<table>
<thead>
<tr>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled Water</td>
<td>0.5 ml</td>
<td>......</td>
<td>......</td>
</tr>
<tr>
<td>Standard</td>
<td>0.5 ml</td>
<td>0.5 ml</td>
<td>......</td>
</tr>
<tr>
<td>TCA</td>
<td>0.5 ml</td>
<td>0.5 ml</td>
<td>......</td>
</tr>
<tr>
<td>Supernatant</td>
<td>......</td>
<td>......</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Urine (1= 48)</td>
<td>......</td>
<td>0.5 ml</td>
<td>......</td>
</tr>
<tr>
<td>Reagent mixture</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
</tbody>
</table>

Mix and let stand for 20 minutes, at 20–25 °C. Measure the absorbance of specimen and standard against reagent blank at 546 nm.

Calculation
Concentration of creatinine in serum:

\[
\text{Creatinine (mg/dL)} = \frac{\text{Aspecimen}}{\text{Astandard}} \times 2
\]

Concentration of creatinine in urine:

\[
\text{Creatinine (mg/dL)} = \frac{\text{Aspecimen}}{\text{Astandard}} \times 2 \times 50
\]

Creatinine clearance:

\[
\text{mg creatinine / dL urine x mL urine / 24 hours} = \frac{\text{UCr x V}}{\text{PCr}} \times \frac{1.73}{\text{A}}
\]

Where:
- \( \text{UCr} \) = Concentration of creatinine in urine (mg/dL)
- \( \text{PCr} \) = Concentration of creatinine in plasma (mg/dL)
- \( \text{V} \) = Volume of urine flow in mL/min.
- \( \text{A} \) = Body surface area in square meter .
- 1.73/A = Factor normalizes clearance for average body surface.

Note : Body surface area can be determined from height weight via normograms in Tietz \((6)\).

Quality Control
Normal & abnormal commercial control serum of known concentrations should be analyzed with each run.

Methods Comparison
A comparison between Spectrum Diagnostics Creatinine colorimetric reagent and acommercial reagent of the same methodology was performed on 40 human sera. A correlation \((r)\) of 0.996 was obtained.

Sensitivity
When run as recommended, the minimum detection limit of the assay is 0.4 mg/dL (0.035 mmol/L).

Linearity
The reaction is linear up to a creatinine concentration of 15 mg/dL; specimens showing higher concentration should be diluted 1+4 using physiological saline and repeat the assay (result \(\times 5\)).

Interfering Substances
Serum, plasma

Haemolysis
Erythrocyte contamination doesn't elevate results.

Icterus
Serum bilirubin levels in the pathological range may interfere with the results.

Lipemia
Lipemic specimens may cause high absorbance flagging. Diluted sample treatment may be recommended.

Expected Values
Serum, plasma

<table>
<thead>
<tr>
<th>Females</th>
<th>0.7-1.3 mg/dL</th>
<th>62-115 mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>0.9-1.5 mg/dL</td>
<td>80-133 mmol/L</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Urine(24 hrs)</th>
<th>Females</th>
<th>0.9 – 1.6 g/24 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>1.1 – 2.8 g/24 hrs</td>
<td></td>
</tr>
</tbody>
</table>

Creatinine clearance

<table>
<thead>
<tr>
<th>Females</th>
<th>75 – 115 mL / min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>85 – 125 mL / min</td>
</tr>
</tbody>
</table>

Dynamic Range
0.4 - 15 mg/dL (0.035 - 1.32 mmol/L).

Waste Disposal
This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.

References

ORDERING INFORMATION

<table>
<thead>
<tr>
<th>CATALOG NO.</th>
<th>QUANTITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>235 001</td>
<td>2 x 100 ml</td>
</tr>
<tr>
<td>235 002</td>
<td>4 x 100 ml</td>
</tr>
<tr>
<td>235 003</td>
<td>8 x 100 ml</td>
</tr>
<tr>
<td>235 004</td>
<td>2 x 500 ml</td>
</tr>
<tr>
<td>235 005</td>
<td>2 x 250 ml</td>
</tr>
</tbody>
</table>

Spectrum Diagnostics does not interpret the results of a clinical laboratory procedure;interpretation of the results is considered the responsibility of qualified medical personnel. All indications of clinical significance are supported by literature references.