INTENDED USE: For the quantitative determination of Creatinine in serum and urine.

CLINICAL SIGNIFICANCE:
Creatinine is synthesized in the body at a fairly constant rate from creatine, which is produced during muscle contractions from creatine phosphate. Creatinine in the blood is then removed by filtration through the glomeruli of the kidney for excretion in the urine. Since the excretion of creatinine in healthy individuals is independent of diet and thus relatively constant, the creatinine clearance (CC) test is one of the most sensitive tests to diagnose renal function especially the glomerular filtration rate (GFR) the concentration of creatinine in serum being dependent almost entirely upon its rate of excretion by the kidney.

Elevated levels of creatinine in serum are usually associated with renal diseases, especially those related to GFR such as glomerular nephritis. Therefore, the clinical significance of the creatinine level in plasma or serum is usually determined in conjunction with the plasma urea level since there is an increase in both levels in post renal azotaemia, while the CC or urine levels, are diminished.

PRINCIPLE:
This procedure is based upon a modification of the original picrate reaction (Jaffe). Creatinine under alkaline conditions reacts with picrate ions forming a reddish complex. The formation rate of the complex measured through the increase of absorbance in a prefixed interval of time is proportional to the concentration of creatinine in the sample.

In End-point Assays, after complete colour development, the absorbance is measured exactly after 30 sec. The colour developed by true creatinine chromogens remains unaffected. Hence this difference in absorbance is directly proportional to the true creatinine concentration.

In Kinetic reactions, the rate of change of absorbance is measured at 520nm at predetermined intervals of time, wherein, the delay time before the formation of the Picrate creatinine complex is monitored. This method is rapid and does not have any deproteinization step.

EXPECTED VALUES:

<table>
<thead>
<tr>
<th>Expected Values</th>
<th>Serum</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>0.6-1.5 mg/dl</td>
<td>1.1-2.0 gm/24 hrs</td>
</tr>
<tr>
<td>Female</td>
<td>0.5-1.2 mg/dl</td>
<td>1.0-1.8 gm/24 hrs</td>
</tr>
</tbody>
</table>

It is recommended that each laboratory establish its own normal range representing its patient population.

KIT CONTENTS:
- PCR 1 (2X50 ml)
- PCR 2 (2X100 ml)
- R1, Picric Acid: 50 ml
- R2, Alkaline Buffer: 50 ml
- R3, Acid Reagent: 5 ml
- R4, Creatinine Standard: 5 ml

(2.0 mg/dl)

SPECIMEN:
Unhemolysed serum/urine

In case of Creatinine Clearance Test, 24-hour urine is preferred. Dilute urine 1:100 with distilled water before use.

WORKING REAGENT PREPARATION:
Mix one volume of Reagent 1 with one volume of Reagent 2 according to the requirement. The Working Reagent is stable for 1 week when stored in dark at RT. All the reagents are stable at room temperature till the expiry date mentioned on the labels.

TEST PROCEDURE:

a) KINETIC METHOD:
Pipette into test tubes labelled Standard (S) and Test (T):

<table>
<thead>
<tr>
<th>Working Reagent</th>
<th>1000 µl</th>
<th>1000 µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>R4 Standard</td>
<td>50 µl</td>
<td>---</td>
</tr>
<tr>
<td>Serum Sample</td>
<td>---</td>
<td>50 µl</td>
</tr>
</tbody>
</table>

b) END POINT METHOD:
Pipette into test tubes labelled Standard (S) and Test (T):

<table>
<thead>
<tr>
<th>Working Reagent</th>
<th>1000 µl</th>
<th>1000 µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Sample</td>
<td>---</td>
<td>100 µl</td>
</tr>
</tbody>
</table>

Mix and keep at 37°C for 5 min. Read absorbance A0, for S and T against distilled water at 520 nm or with green filter (505-570 nm).

R3 Acid Reagent: 50 µl
Mix and keep at RT for 5 min. Read absorbance A1 for S and T against distilled water at 520 nm or with green filter (505-570 nm).

Determine ∆A for S and T

∆A = AT - A0

CALCULATIONS:

a) Serum Creatinine in mg/dl

\[ \text{Serum Creatinine} = \frac{\Delta A x 2}{\Delta AS} \]

b) Urine Creatinine in gm/L

\[ \text{Urine Creatinine} = \frac{\Delta A x 2}{\Delta AS} \]

c) Urine Creatinine in gm/24 hrs.

\[ \text{Urine Creatinine} = \frac{\text{b} x 24 \text{ hrs Urine volume} \text{ collected in litres}}{1000} \]

QUALITY CONTROL:
To ensure adequate quality control, the use of commercial reference control serum is recommended with each assay batch. Use of quality control material checks both the instrument and reagent functions.

PRECISION:
Precision studies were performed with two controls using NCCLS protocol EPS-A. The results of the precision studies are shown below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Within-run</th>
<th>Between-run</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>CV%</td>
<td>Mean</td>
</tr>
<tr>
<td>Control 1</td>
<td>2.02</td>
<td>1.25</td>
<td>5.76</td>
</tr>
<tr>
<td>Control 2</td>
<td>5.56</td>
<td>2.15</td>
<td>7.62</td>
</tr>
</tbody>
</table>

LINEARITY:
The procedure is linear up to 20 mg/dl. If values exceed this limit, dilute the sample with normal saline and repeat the assay. Calculate the value using the proper dilution factor.

SYSTEM PARAMETERS:

<table>
<thead>
<tr>
<th>Reaction type</th>
<th>: Fixed Time</th>
<th>Blank : D.Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wave length</td>
<td>: 520 nm (505-570)</td>
<td>Standard : 2.00</td>
</tr>
<tr>
<td>Flow Cell Temp</td>
<td>: 37°C</td>
<td>Units : mg/dl</td>
</tr>
<tr>
<td>W.Reagent vol.</td>
<td>: 1000 µl</td>
<td>Sample vol. : 50 µl</td>
</tr>
<tr>
<td>Delay Time</td>
<td>: 30 Sec</td>
<td>Fixed Time : 90 Sec</td>
</tr>
<tr>
<td>Low Normal</td>
<td>: 0.6</td>
<td>High Normal : 1.5</td>
</tr>
</tbody>
</table>

NOTE:
1. Adherence to the reaction time should be meticulously followed.
2. The normal range is approximate and varies with the sex and body weight.
3. In the End-Point method after addition of Acid Reagent in Test, there may be slight haziness, which disappears on thorough mixing.

REFERENCES: