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 picate reaction (Jaffe). Creatinine under alkaline conditions reacts with picate 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 Kinetic reactions, the rate of change of absorbance is measured at 520 nm 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 deproteini zation.

EXPECTED VALUES:

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

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

KIT CONTENTS:
PCLR 1 (2X50 ml)
1. Creatine Reagent 2x 50 ml
2. Creatinine Standard (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.

STORAGE & STABILITY:
All the reagents are ready to use and are stable at 2-8°C till the expiry date mentioned on the labels.

TEST PROCEDURE:
Pipette into test tubes labelled Standard (S) and Test (T):

<table>
<thead>
<tr>
<th></th>
<th>($)</th>
<th>(T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine Reagent</td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
<tr>
<td>Standard</td>
<td>100 µl</td>
<td>---</td>
</tr>
<tr>
<td>Serum Sample</td>
<td>---</td>
<td>100 µl</td>
</tr>
</tbody>
</table>

Reaction Temperature: 37°C
Mix well and read absorbance of S and T against distilled water at 520 nm (505-570) as follows:
Initial absorbance \( A_0 \) - Exactly after 30 sec.
Final absorbance \( A_f \) - Exactly 90 sec After \( A_0 \).
Determine \( \Delta A \) for S and T
\[ \Delta AS = AS_f - AS_0 \]
\[ \Delta AT = AT_f - AT_0 \]

CALCULATIONS:
a) Serum Creatinine in mg/dl \( = \frac{\Delta AT}{\Delta AS} \times 2 \)
b) Urine Creatinine in gm/L \( = \frac{\Delta AT}{\Delta AS} \times 2 \)
c) Urine Creatinine in gm/24 hrs. \( = b \times 24 \text{ hrs.} \) Urine volume to be collected in litres.

QUALITY CONTROL: To ensure adequate quality control, the use of 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 EP5-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>2.12</td>
</tr>
<tr>
<td></td>
<td>4.14</td>
<td>3.50</td>
<td></td>
</tr>
<tr>
<td>Control 2</td>
<td>5.56</td>
<td>2.15</td>
<td>5.76</td>
</tr>
<tr>
<td></td>
<td>11.32</td>
<td>5.30</td>
<td></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:
Reaction type: Fixed Time/Two Point Kinetic
Blank: D.Water
Wave length: 520 nm (505-570)
Flow cell Temp: 37°C
Reagent vol: 1000 µl
Sample vol: 100 µl
Delay Time: 30 Sec
Low normal: 0.6
High normal: 1.5

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.

REFERENCES: