PROCEDURE:
Pipette into clean dry test tube labeled as Standard (S) and Test (T):

<table>
<thead>
<tr>
<th>Addition sequence</th>
<th>S</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working reagent</td>
<td>1 ml</td>
<td>1 ml</td>
</tr>
<tr>
<td>Standard</td>
<td>10 µl</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>10 µl</td>
</tr>
</tbody>
</table>

Mix well and read the initial absorbance A for the Standard and the Test after exactly 30 seconds. Read another absorbance A after exactly 60 seconds later. Calculate the change in absorbance \( \Delta A \) for both the Standard and Test.

For Standard: \( \Delta AS = AS_2 - AS_1 \)

For Test: \( \Delta AT = AT_2 - AT_1 \)

CALCULATION:

Urea (mg/dl) = \( \frac{\Delta AT}{50} \) \\

NORMAL VALUE:
Serum / Plasma: 10-40 mg/dl
Urine: 26 to 43 g/24 hrs.

Urea/Creatinine ratio:
25:40 [(mmol/L)/(mmol/L)]
20:35 [(mg/dL)/(mg/dL)]

LINEARITY:
The procedure is linear up to 300 mg/dl. If the value exceeds this limit, dilute the serum with normal saline (NaCl 0.9%) and repeat the assay. Multiply result by dilution factor.

QUALITY CONTROL:
For accuracy it is necessary to run known controls with every assay.

LIMITATION & PRECAUTIONS:
The reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes. The use of the laboratory reagents according to good laboratory practice is recommended.

REFERENCES:
(3) Talke H, Schubert GE. Enzymatische Harnstoff-bestimmung in Blut und Serum im optischen Test nach Warburg (Enzymatic determination of urea in blood and serum with the optical test according to Warburg). Klin Wschr 1965; 43:174-5.