Tests for evaluation of kidney functions

1. Urea
2. Uric acid
3. Creatinine
Basic steps in urine formation

1. Filtration
2. Reabsorption (solute, water)
3. Secretion (additional wastes)
4. Excretion
Renal dysfunction

Excretion of Urea / Uric acid / Creatinine in urine

Hyperuremia

Hyperuricemia

Serum Creatinine levels
1. Urea:

\[ \text{H}_2\text{N-C-}\text{NH}_2 \]

- In human, Urea is the main end product of protein and amino acids catabolism.
Dietary Proteins

Digestion

Amino acids

Tissue protein (aminoacid pool)

Kidney excretion

<table>
<thead>
<tr>
<th>COOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
</tr>
<tr>
<td>NH₂</td>
</tr>
<tr>
<td>R</td>
</tr>
</tbody>
</table>

\[ \text{R-CO}_2H + \text{NH}_3 \rightarrow \text{RCO}_2\text{NH}_2 \]

\[ \text{RCO}_2\text{NH}_2 \rightarrow \text{RCO}_2\text{H} + \text{NH}_3 \]
Determination of serum urea concentration by urease method:

**Principle:**

* \( \text{NH}_2\text{-CO-NH}_2 + \text{H}_2\text{O} \xrightarrow{\text{Urease}} 2 \text{NH}_3 + \text{CO}_2 \)  

* \( \text{NH}_3 + \text{NaOCl} \xrightarrow{} \text{NH}_2\text{Cl} + \text{H}_2\text{O} \)  

* \( \text{NH}_2\text{Cl} + \text{Sod. salicylate} \xrightarrow{\text{NaOH}} \text{Green complex} \)  

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* **Sodium salicylate:** coloring reagent  
* **Nitroprusside:** acts as a catalyst, the intensity of the colour.
### Procedure:

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Working Solution</strong></td>
<td>1 ml</td>
<td>1 ml</td>
<td>1 ml</td>
</tr>
<tr>
<td>(Urease + buffer pH 8 + Na</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salicylate + Na nitroprusside)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Serum</strong></td>
<td>------</td>
<td>--------</td>
<td>0.1 ml</td>
</tr>
<tr>
<td><strong>Standard</strong></td>
<td>------</td>
<td>0.1 ml</td>
<td>------</td>
</tr>
<tr>
<td><strong>Alkaline Reagent</strong></td>
<td>0.2 ml</td>
<td>0.2 ml</td>
<td>0.2 ml</td>
</tr>
<tr>
<td>(NaOH + NaOCl)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Mix, leave for 5 min at room temperature**

**Mix, leave for 10 minutes at room temperature**

**Measure the absorbance at 578 nm**
Calculation:
\[
\text{Conc. of urea} = \frac{\text{Abs. of test}}{\text{Abs. of standard}} \times \text{Conc. Of standard}
\]

Normal range: 14 – 50 mg/dl (mg %)

Interpretations: *Physiologically:

- Urea α amount of protein in diet.
- Serum urea is lower (15 to 20 mg/dl) in late pregnancy due to hemodilution and fetus consumes amino acids.
- It increases by age due to increase the rate of protein catabolism.
*Pathologically:*

1- Serum urea **↓** in severe liver diseases.
2- Serum urea **↑** in kidney diseases.
2- Uric acid (urate):

-In man, uric acid is the end product of purine catabolism and is excreted by the kidney.

-Purine bases are Adenine and Guanine which forms nucleotides of DNA and RNA.
- Uric acid is **sparingly** soluble in water.

- In **animals**:
  
  Uric acid $\xrightarrow{\text{uricase}}$ Allantoin

- **Human**: lack uricase enzyme so:
  
  • Blood can dissolve a limited amount of uric acid (up to 7 mg/dl) in the form of sodium urate.
In hyperuricemia:

- Uric acid increases in blood exceeding the soluble amount

   - Precipitate as needle crystals in joints causing severe pain and inflammation

   gout (gouty arthritis)
Principle:

* Uric acid + O_2 + H_2O \rightarrow \text{Allantoin} + \text{CO}_2 + \text{H}_2\text{O}_2

* H_2O_2 + O-acceptor (4-aminophenazone) + coloring reagent (Phenol) \rightarrow \text{Colored Rose product}
**Procedure:**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Blank</th>
<th>Standard</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>------</td>
<td>---------</td>
<td>0.1 ml</td>
</tr>
<tr>
<td>Standard</td>
<td>------</td>
<td>0.1 ml</td>
<td>----------</td>
</tr>
<tr>
<td>Working reagent</td>
<td>1 ml</td>
<td>1 ml</td>
<td>1 ml</td>
</tr>
</tbody>
</table>

Mix and leave for **10 min. at room temperature** and measure absorbance of Test & Std. at 520 nm.
Calculation:

\[
\text{Conc. of uric acid (mg/dl)} = \frac{\text{Abs. of } T}{\text{Abs. of } St.} \times \text{Conc. of st.}
\]

Normal range:

- For female: 2 – 6 mg/dl
- For male: 3 – 7 mg/dl

Interpretation:

Uric acid (hyperuricemia): gout
Thank You