Estimation of Uric acid in serum

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Crystals of urate in polarized light
• Uric acid is the end product of purine catabolism.
• Formation of uric acid is principally endogenous mainly of tissue nucleoprotein breakdown but some amount is also formed from purine containing compounds present in food.
• The serum uric acid levels are affected by diet.
Most uric acid is dissolved in the blood, filtered through the kidneys, and expelled in the urine. Sometimes, the body produces too much uric acid or does not filter out enough of it.
Uric acid is a chemical produced when your body breaks down foods that contain organic compounds called purines. These foods include liver, anchovies, mackerel, dried beans, beer, and wine. Purines are also created through the natural process of cell breakdown in the body.

<table>
<thead>
<tr>
<th>High Purine Foods Source</th>
<th>Low Purine Foods Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef</td>
<td>Milk, cheese, eggs, nuts, fruits, vegetables</td>
</tr>
<tr>
<td>Salmon</td>
<td></td>
</tr>
<tr>
<td>Chicken</td>
<td></td>
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<tr>
<td>Sausages</td>
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<tr>
<td>Mushrooms</td>
<td></td>
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<tr>
<td>Liver</td>
<td></td>
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<tr>
<td>Anchovies</td>
<td></td>
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<tr>
<td>Mackerel</td>
<td></td>
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<tr>
<td>Dried beans</td>
<td></td>
</tr>
<tr>
<td>Beer</td>
<td></td>
</tr>
<tr>
<td>Wine</td>
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</tbody>
</table>
Abnormalities

• **Hyperuricemia** is the disorder that occurs when you have too much uric acid in your body.

• **Hypouricemia** is the too little uric acid in your blood, which is a symptom of a disorder of the kidney tubules that prevents the absorption of substances such as glucose and uric acid, which are then passed in the urine.

Normal range= 2-7 mg/dl
Purposes of a Uric Acid Blood Test

• diagnose and monitor patients with **gout**
• check kidney function post-injury
• determine the cause of kidney stones
• diagnose kidney disorders
High levels of uric acid in may suggest:

- gout (recurring attacks of acute arthritis)
- chemotherapy
- bone marrow disorders (such as leukemia)
- a diet high in purines
- kidney disorders
- kidney stones
Low levels of uric acid in the blood may suggest:

- Fanconi syndrome (a kidney tube disorder)
- Alcoholism
- Liver or kidney disease
- A diet low in purines
Uric acid Structure

Chemically uric acid is 2, 6, 8 trihydroxypurine.

Chemical formula $\text{C}_5\text{H}_4\text{N}_4\text{O}_3$
• It acts like a dibasic acid and can form mono and disodium salts depending on the pH.
• Only pH of 5.75 is possible inside body such as in renal tubules.
• At this pH, or above it exists as monosodium urate salt.
• Thus in plasma, it is mainly as monosodium urate.

\[
\text{monosodium urate.}
\]
Objective:

To measure the amount of uric acid in blood
Principle:

1-Uric acid in the sample oxidized by uricase to allantoin and hydrogen peroxide.

2-Hydrogen peroxide reacts with polyhalogenated benzoic acid (PHBA) and 4-aminoantipyrine (4-AAP) in the presence of peroxidase (Hydrogen peroxide oxidoreductase) to yield a quinoneimine dye (chromogen). The intensity of the dye is measured and is directly proportional to the concentration of uric acid present in the sample.

\[
\text{Uric acid} + \text{O}_2 + 2\text{H}_2\text{O} \xrightarrow{\text{Uricase}} \text{Allantoin} + \text{CO}_2 + \text{H}_2\text{O}_2 \\
2\text{H}_2\text{O}_2 + 4\text{-AAP} + \text{PHBA} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine (chromogen)} + 4\text{H}_2\text{O}
\]
Materials:

• REAGENT COMPOSITION

• **URIZYME BUFFER**: Polyhalogenated benzoic acid in Tris buffer at pH 7.5 ± 0.05.

• **URIZYME REAGENT**: 4-Aminoantipyrine, Peroxidase, Uricase

• **URIC ACID STANDARD** (5 mg/dL): An aqueous solution containing 5 mg/dL Uric acid.

• *Tow Serum sample*
**Method:**

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urizyme buffer</td>
<td>1 ml</td>
<td>1 ml</td>
<td>1 ml</td>
</tr>
<tr>
<td>Urizyme Reagent</td>
<td>0.1 ml</td>
<td>0.1 ml</td>
<td>0.1 ml</td>
</tr>
<tr>
<td>Standard</td>
<td>—</td>
<td>0.025 ml</td>
<td>—</td>
</tr>
<tr>
<td>Sample</td>
<td>—</td>
<td>—</td>
<td>0.025 ml</td>
</tr>
</tbody>
</table>

- Water bath at 37°C for 5 min
- Read absorbance at 510 nm
Calculations:

Concentration of uric acid in normal serum sample1 = 
\[(\text{absorbance of sample} \div \text{absorbance of standard}) \times 5\] = mg/dl

Concentration of uric acid in abnormal serum sample2 = 
\[(\text{absorbance of sample} \div \text{absorbance of standard}) \times 5\] = mg/dl

RANGE OF EXPECTED VALUES IN SERUM
3.4 - 7.0 mg/dL
Discussion

Comment on your result and mention if there are any abnormalities
References

• http://www.healthline.com/health/uric-acid-blood

• URIC ACID (TRINDER) W/BUFFER ENZYMATIC COLOR/ END POINT METHOD Kit from UDI
Questions

• What is the chemical composition of gout deposits/tofhus?
• What the mechanism of action of febuxostat and allopurinol?
Thank You