Estimation of Uric Acid in serum
Synthesis of uric acid

Nucleic acid

Adenine (6-amino purine)

Guanine (2-amino, 6-oxy purine)

Hypoxanthine (6-oxy purine)

Xanthine (2, 6-dioxo purine)

Uric acid (2, 6, 8-trioxo purine)
Uric acid Structure

Chemically uric acid is 2, 6, 8 trihydroxypurine.
Forms of Uric Acid

• It is a weak organic acid that under physiologic conditions exists mainly as a monosodium salt.

• At a pH less than 5.75, as may occur in the urine, the predominant form is nonionized uric acid.

• The solubility of monosodium urate is about 18 times greater than uric acid in aqueous solutions. This solubility differential provides the therapeutic rationale for alkalinization of the urine pH to greater than 6.0 in patients forming uric acid stons.
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.
Sources of Uric Acid

• Uric acid is a chemical produced when body breaks down foods that contain organic compounds called purines. These foods include liver, meat, dried beans, beer, and wine.

• Purines are also created through the natural process of cell breakdown in the body.

• 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.
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
Blood
Higher than normal uric acid levels in the blood is called hyperuricemia and can be caused by the overproduction of uric acid in the body or the inability of the kidneys to adequately remove enough uric acid from the body. Further investigation is needed to determine the cause of the overproduction or decreased excretion of uric acid.

hyperuricemia can cause solid crystals to form within joints. This causes a painful condition called gout. If gout remains untreated, uric acid crystals can build up in the joints and nearby tissues, forming hard lumpy deposits called tophi. High levels of uric acid in the urine can cause kidney stones.
Abnormalities

• **Urine:**

  The uric acid **urine test** measured in a sample of urine collected over 24 hours.

  Most of the uric acid is removed from the body in urine. A small amount passes out of the body in stool. But if too much uric acid is being produced, the level in the urine will increase. If the **kidneys are not able to remove it from the blood** normally, the level of **uric acid in the urine will decrease**.

  High uric acid levels in the urine are seen with gout, multiple myeloma, metastatic cancer, leukemia, and a diet high in purines. Those at risk of kidney stones who have high uric acid levels in their urine may be given medication to prevent stone formation.

• **Low** urine uric acid levels may be seen with **kidney disease, chronic alcohol use, and lead poisoning**.
Figure 1 Metabolism of uric acid and risk factors for gout

- High-purine diet
- Dietary purines
- Purine nucleotides and bases
- Tissue nucleotide synthesis and metabolism

30% 
70% 

Uric acid pool (serum, tissues)

70% renal excretion
30% gut elimination

- Genetic factors
- Metabolic syndrome (hypertension, insulin resistance, hyperlipidaemia)
- Renal impairment
- Drugs that reduce renal function

Obesity

MSU crystals

Osteoarthritis Ageing

Rees, F. et al. (2014) Optimizing current treatment of gout
Nat. Rev. Rheumatol. doi:10.1038/nrrheum.2014.32
Gout

• Gout is a group of diseases caused by an increased conversion of purine bases to uric acid or a decreased excretion of uric acid by the kidney.

• An accumulation of uric acid, which is very insoluble, results in the precipitation of urate crystals in the joints. An acute inflammatory arthritis results. **Chronic cases are treated with allopurinol, that inhibits xanthine oxidase and prevents hypoxanthine and xanthine from being converted to uric acid.**

Allopurinol (AP), which inhibits xanthine oxidase, is used to treat gout. Gout occurs when uric acid crystals precipitate in joints because of an increased concentration in the blood.
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.

Uric acid + O2 +2H2O $\xrightarrow{\text{Uricase}}$ Allantoin + CO2 + H2O2-

2H2O2- +4-AAP + PHBA $\xrightarrow{\text{Peroxidase}}$ Quinoneimine +4H2O

(chromogen)
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.

- **Serum sample**
Method:

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<tr>
<td>Sample</td>
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<td>0.025 ml</td>
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-Water bath at 37°C for 5 min
-Read absorbance at 510 nm
Calculations:

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

- URIC ACID (TRINDER) W/BUFFER ENZYMATIC COLOR/ END POINT METHOD Kit from UDI
- BRS Biochemistry, Molecular Biology & Genetics, Sixth Edition
Questions

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