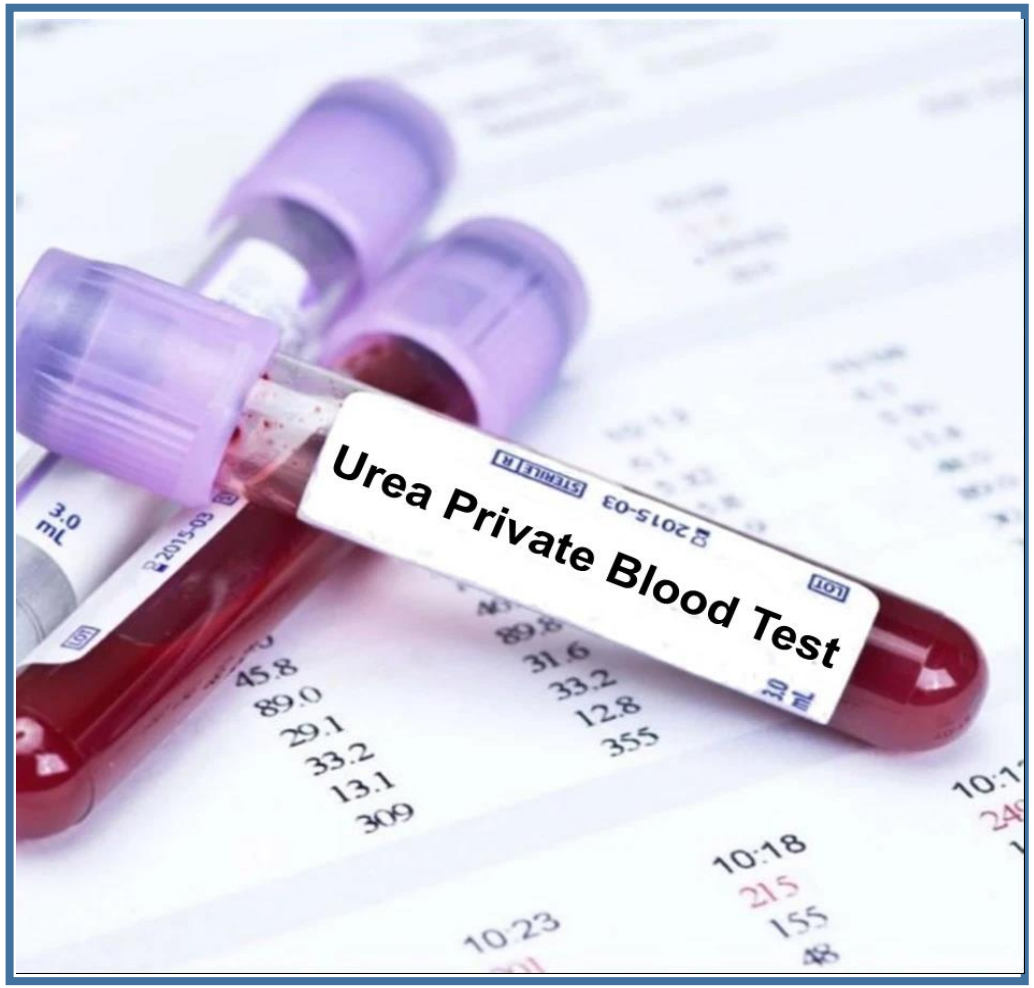

Quantitative Determination of Blood Uric Acid

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Quantitative Determination of Blood Uric Acid

Introduction

Quantitative Determination of Blood Uric Acid is considered one of the most commonly performed procedures in clinical biochemistry laboratory. Uric acid is a heterocyclic compound with the formula $C_5H_4N_4O_3$. It forms ions and salts called urates or acid urates such as ammonium acid urate. It is a product of metabolic breakdown of purines and it is a normal component of the urine. Hyperuricemia is the abnormal elevated levels of uric acid in blood and could lead to gout and associated with other medical conditions including DM, formation of kidney stones and cardiovascular disease (CVD).

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Clinical Significance

Uric acid is the major product of the catabolism of the purines. Major sources of hyperuricemia are:

1- Primary gout: due to metabolic overproduction of purines or under-excretion of uric acid.
2- Secondary gout: due to renal disease.
3- Administration of some drugs such as thiazide diuretics.
4- Primary defects of enzymes in the pathway of purines metabolism.
5- Fasting or rapid weight loss.
6- High dietary purines rich foods such as meats, high fructose corn syrup and table sugar.
7- reduced excretion of urine by the kidneys.
8- Tumor lysic syndrome (TLS) which is a group of metabolic abnormalities that can occur as a complications during treatment from cancer by chemotherapeutic agents.

From the other hand, hypouricemia (the abnormal low levels of uric acid in blood) is caused by low dietary zinc and oral contraceptive medication and could lead to multiple sclerosis. It could be treated by increasing zinc dietary intake.

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Sample Handling

Samples for uric acid analysis should be collected from the patients in fasting state (No eat or drink for 12 hrs) and delivered to the laboratory as soon as possible after being drawn from the patient to separate the blood serum or heparinized plasma for analysis (Unhemolysed serum or plasma in heparin or EDTA tubes).

Uric acid in the blood serum is stable for 3 days at room temperature, 7 days at 2-8 °C and 6 months at -20°C but repeated freezing and thawing should be avoided.

Interferences

High bilirubin or ascorbic acid (Vit.C) levels may result in negative interference. Grossly lipemic or hemolysed specimen can cause falsely increase in uric acid value.

In order to reduce Vit.C interference let stand the specimen 2 hrs at room temperature before performing the assay.

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Principle

Uricase acts on uric acid to produce allantoin, carbon dioxide and hydrogen peroxide. Hydrogen peroxide in the presence of peroxidase reacts with (amino-antipyrine and dichlorohydroxybenzene sulfonate) to yield quinoneimine (red color complex). The absorbance is proportional to the amount of uric acid in the specimen.

$$\text{Uric acid} + O_2 + H_2O \xrightarrow{\text{Uricase}} \text{Allantoin} + H_2O_2 + CO_2$$

$$H_2O_2 + \text{amino-antipyrine} + \text{dichlorohydroxybenzene sulfonate} \xrightarrow{\text{Peroxidase}} \underline{\text{Quinoneimine}}$$

Reagents

1- Enzyme Reagent (Vial R1): Uricase ≥ 120 U/L, Potassium hexacyanoferrate (II) 42 µmol/L, Peroxidase ≥ 450 U/L, Amino-antipyrine 0.150 mmol/L.

2- Buffer (Vial R2): Dichlorohydroxybenzene sulfonate 2 mmol/L, Tris pH=8 @ 25°C 50 mmol/L, Preservative.

3- Standard Concentration Solution of Uric acid (10 mg/dL).

Reagent Preparation

Working Reagent Solution was prepared by mixing and dissolving the content of Vial R1 in the content of Vial R2 and store it at 2-8 °C away from light.

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Procedure

1- Collect blood specimen from patients in serum blood tubes and leave it to coagulate for 20 minutes.
2- Separate serum sample from the blood by centrifuging at 3000-4000 RPM for 10-20 minutes.
3- Collect the serum in labeled tubes.
4- Three sets of tubes were prepared as below:

Solutions / Tubes	Blank	Standard	Serum
Working Reagent	**1 mL**	**1 mL**	**1 mL**
Standard	**-**	**25 µL**	**-**
Sample (Serum)	**-**	**-**	**25 µL**

5- All tubes were mixed well by vortex and incubated them for 5 minutes at 25ºC.
6- The absorbance (A) of the serum samples and standard were measured against the blank at wave length 520nm by using cuvette of 1 cm light path of Sepctrophotometer.

** **Advantage of Blank Tube is to zero the instrument and eliminate all the effects of light (like refraction, diffraction, ..etc), measurement tube material and all other compounds in the solution except the target compound.**

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Calculations

$$\text{Level of Uric acid (mg/dL)} = \frac{\text{A (Sample)}}{\text{A (Standard)}} \times N$$

Where: N = Concentration of uric acid in standard solution = 10 mg/dL.

Reference Range

Normal Uric acid Level in Serum (Children) = 2 – 5.5 mg/dL (Higher in newborns).

Normal Uric acid Level in Serum (Men) = 3.5 – 7.2 mg/dL.

Normal Uric acid Level in Serum (Women) = 2.6 – 6 mg/dL (Lower during pregnancy).