Uric Acid

(Phosphotungstate Method)

Code : 10023 (3 x 100 ml)

(For the Analyser/Colorimetric Estimation of Uric Acid in Serum / Plasma)
In VITRO USE Only.

SUMMARY & EXPLANATION OF TEST:

In human beings, uric acid is the end product of purine metabolism. Although uric acid is a non-protein nitrogen substance, its determination has a diagnostic value in differentiating gout from non-gout arthritis. Most of the methods for uric acid estimation are based on the oxidation of uric acid in alkaline medium by phosphotungstate reagent which itself gets reduced to tungsten blue. Two errors are common in uric acid estimation, some uric acid is carried down with the protein precipitate giving low results while some substances present in blood may also give a color, leading to high results. Such substances are mostly found in red blood cells, so that error due to them can be largely eliminated by using serum or plasma.

Uric acid kit is based upon the reduction of phosphotungstate reagent in alkaline medium. Tungstic acid reagent is used for deproteinization of specimen. Tungstic acid is usually unstable, however EXCEL Tungstic acid reagent is specially stabilized.

PRINCIPLE:

Tungstic acid precipitates serum plasma proteins with the least removal of uric acid along with the protein precipitate. In alkaline medium uric acid reduces phosphotungstate reagent producing tungsten blue color, whose absorbance is proportional to uric acid concentration.

REAGENTS:

1.	Tungstic Acid Reagent	100 ml
2.	Sodium Carbonate 14% W/V	100 ml
3.	Phosphotungstate Reagent	100 ml
4.	Uric Acid Standard stock (100 mg%)	3 ml

The reagents 1 and 2 are to be stored at room temperature, while reagents 3 and 4 are to be stored at 2-8^{o}C. All the reagents are stable till the expiry date mentioned on the individual label.

REAGENT PREPARATION :

Dilute 0.1 ml of uric acid stock standard (4) to 10 ml with distilled water and mix well. Prepare fresh working standard everyday.

SAMPLE :

Serum / Plasma can be used. In case of plasma any of the common anticoagulants except potassium oxalate can be used. Potassim oxalate forms insoluble potassium phosphotungstate resulting in turbidity. RBCs contain sulfhydryl compounds which give false high results, therefore the serum should be unhemolysed.

EXPECTED RANGE:

Male	:	2.5 to 7.0 mg%
Female	:	1.5 to 6.0 mg%

LINEARITY:

upto	:	20 mg%

INSTRUCTIONS:

1. The filtrate supernatant during deproteinization of the specimen should be crystal clear.

DIRECTIONS FOR USE ON ANALYSERS:

Reaction Type	:	End point with standard
Reaction Slope	:	Increasing
Wave Length	:	710 nm (Red filter)
Incubation Temp	:	Dark
Incubation Time	:	15 min.
Standard	:	10 mg%
Linearity	:	20 mg%
Unit	:	mg%

PROCEDURE :

I. Step 1:

Pipette into clean dry test tubes labeled (T).

	(T)
Serum/plasma	0.5 ml
Tungstic Acid Reagent	3.0 ml
Distilled Water	1.5 ml

Mix well and keep it for 10 minutes at RT. Centrifuge or filter through Whatmann No. 1 filter paper.

II. Step 2:

	(B)	(S)	(T)
Supernatant/filtrate From Step 1	--	--	3.0 ml
Working Standard	--	3.0ml	--
Distilled Water	3.0ml	--	--
Sodium Carbonate (2)	1.0ml	1.0ml	1.0ml
Phosphotungstate (3)	1.0ml	1.0ml	1.0ml

Mix well and incubate in dark for 15 minutes and measure the absorbance of Standard (S) and Test (T) against Blank (B) either on a photocolorimeter with redfilter or on spectrophotometer at 710 nm.

CALCULATIONS:

$$\text{Uric acid concentration in mg \%} = \frac{\text{A of (T)} \times 10 \text{ (Std. Conc)}}{\text{A of (S)}}$$

SI Conversion factor mmol/L = mg% x 0.0595

NOTES:

★ Due to variations in inter - laboratory assay conditions, instruments and demography, it is recommended that each laboratory should establish its own normal range. To ensure adequate quality control, each run should include a normal and abnormal assayed controls. The assigned value of the control must be confirmed by this methodology.

★ Final diagnosis should be based on a co-relation of test results with other clinical observations / Diagnostic tools.

BIBLIOGRAPHY:

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