URIC ACID (Uricase)

INTENDED USE

Bioline Uric acid is used for the quantitative determination of uric acid in serum.

CLINICAL SIGNIFICANCE

Uric acid is the end product of purine metabolism. Nearly half of the total uric acid is eliminated and replaced each day by way of urinary excretion and through microbial degradation in the intestinal tract. Increased uric acid levels are commonly associated with both nitrogen retention and urea, creatine, and other non-protein constituents. The quantitation of uric acid is an aid in the diagnosis of gout, decreased renal function, myelo proliferative disorders, and other conditions in which the cause for the hyper- uricemia is not well known.

METHOD AND PRINCIPLE

Uric acid is most commonly determined by a phosphotungstate method and iron reduction method. Due to serum interferences, the enzyme uricase has been widely used instead. Uricase is more specific for uric acid since uricase acts only on uric acid.

The enzymatic reaction sequence employed in the assay of uric acid is as follows:

$$\text{Uricacid} + O_2 + 2H_2O \xrightarrow{\text{Uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

$$2H_2O_2 + \text{4-Aminoantipyrine} + \text{DHBS} \xrightarrow{\text{Peroxidase}} \text{Chromogen} + 4H_2O$$

Uric Acid is converted by uricase into allantoin and hydrogen peroxides. The hydrogen peroxide initiates the coupling of 4-aminoantipyrine to 3,5-dichloro-2-hydroxy benzene sulfonic acid (DHBS) to form the chromogen which is measured at 505 nm and which is proportional to the amount of hydrogen peroxide generated from uric acid.

REAGENT COMPOSITION

1. Uric acid reagent: 4-Aminoantipyrine 4mM,3,5 Dichloro-2-hydroxy benzene sulfonate 2mM,Stabilizer and Surfactant, buffer pH 7.5.
2. Uric acid standard (6 mg/dl).

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use.
   CAUTION: The reagents may be hazardous. Handle in accordance with good laboratory procedures, which dictate avoiding ingestion, and eye or skin contact.
2. Serum specimens should be considered infectious and handled appropriately.

REAGENT PREPARATION

Reagent and standard are ready to use.

REAGENT STORAGE AND STABILITY

The reagent set is stored refrigerated (2 - 8°C).DO NOT FREEZE. Bring reagent to room temperature before use.

REAGENT DETERIORATION

The reagent should be discarded if:

1. Turbidity has occurred; turbidity may be a sign of contamination.
2. There is evidence of discoloration. As light pink color is normal.

SPECIMEN COLLECTION AND STABILITY

1. Test specimen should be serum and free from hemolysis.
2. Bacterial contamination should be avoided to preserve the loss of uric acid.
3. Uric acid in serum is stable for 3 days at 2- 8°C and upto 6 months when frozen.

INTERFERENCES

1. Bilirubin and ascorbic acid can result in falsely depressed uric acid levels.
2. Lipemic samples may cause falsely elevated uric acid levels.
3. Collection tubes containing formaldehyde as a preservative must be avoided.
4. For a comprehensive review of drug interferences refer to Young et al.

ASSAY PROCEDURE FOR SEMIAUTO ANALYZER

Wavelength : 505 nm
Temperature: 37°C

	Blank	Standard	Sample
Reagent	1000 µL	1000 µL	1000 µL
Distilled water	25 µL	-	-
Standard	-	25 µL	-
Sample	-	-	25 µL

Mix and read optical density of standard and sample against reagent blank after 5 min of incubation at 37°C or 10 min at RT.

CALCULATIONS

A= Absorbance

$$\frac{A\ \text{unknown}}{A\ \text{Standard}} \times \text{Standard Concn (mg/dl)} = \text{Uric acid(mg/dL)}$$

Example: If the unknown A = 0.170, standard A = 0.180, concentration standard = 6 mg/dl, then:

$$\text{Uric acid (mg/dl)} = \frac{0.170}{0.180} \times 5 = 5.6\ \text{mg/dl}$$

CALIBRATION

The procedures are calibrated with the standard solution, which is included with each series of tests. Its absorbance is used to calculate the results. It is recommended to establish a linearity curve up to 25 mg/dl with other available commercial standard solutions to verify the performance of instruments and reagents.

Factor above 34 indicated standard deterioration in these cases multiply the absorbance with fix factor of 29 to get the sample results.

LIMITATIONS

The reagent is linear upto 25mg/dl uric acid. Samples with values exceeding 25 mg/dl should be diluted 1:1 with saline, re assayed and the results multiplied by 2. Lipemic samples will give falsely elevated results and a serum blank must be run.

Serum blank: Add 0.025 ml (25 µl) of sample to 1.0 ml water. Zero the spectrophotometer with water. Read and record absorbance and subtract reading from test absorbance.

QUALITY CONTROL

It is recommended that controls be included in each set of assays. Commercially available control material with established uric acid values may be used for quality control. The assigned value of the control material must be confirmed by the chosen application. Failure to obtain the proper range of values in the assay of control material may indicate reagent deterioration, instrument malfunction, or procedural errors.

EXPECTED VALUES

1.5-7.0 mg/dl

It is strongly recommended that each laboratory establish its own normal range.

PERFORMANCE CHARACTERISTICS

1. Linearity: 25 mg/dl
2. Comparison: A comparison with another commercial enzymatic uric acid procedure yielded a correlation coefficient of 1.00 with a regression equation of y= 1.02 x - 0.22.

General Technical Parameters

Mode	**End Point**
Wavelength (Filter)	**505 nm**
Reaction Direction	**Increasing**
Reagent Blank	**Yes**
Sample Vol.	**25 µL**
Reagent Vol.	**1000 µL**
Incubation Time	**5 min**
Reagent Blank Abs. (Max.)	**NMT 0.300 Abs**
Calibration Method	**1- Point**
Standard (Conc.)	**6.0 mg/dL**
Linearity	**25.0 mg/dL**
Decimal Places	**1**
Temp.	**37 °C**
Unit	**mg/dL**
Ref. Low (Male / Female)	**1.5 mg/dL**
Ref. High (Male / Female)	**7.0 mg/dL**

REFERENCES

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