

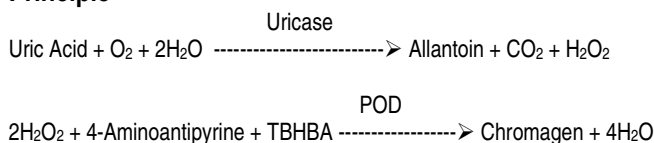
### Intended Use

For the quantitative determination of Uric Acid in serum.

### Method History

Uric Acid has been determined by phosphotungstate methods,<sup>1</sup> variations of the phosphotungstate method<sup>2</sup> and iron reduction methods.<sup>3,4</sup> The above methodologies are influenced by many substances in their procedures as well as many contaminating substances on glassware, etc.<sup>5</sup> The enzyme Uricase has been widely used for Uric Acid determinations because of its improved specificity.<sup>6,7</sup> Recently, hydrogen peroxide, a by-product of the uricase/uric acid reaction, has been coupled to other enzymatic reactions to yield a colorimetric end product. The present procedure uses the coupling of 4-aminoantipyrine with 2-Hydroxy-2,4,6-tribromobenzoic acid (TBHBA) and hydrogen peroxide in the presence of peroxidase to yield a chromagen measured at 520nm.

### Principle



Uric Acid is oxidized by Uricase to allantoin and hydrogen peroxide. TBHBA + 4-aminoantipyrine + hydrogen peroxide, in the presence of peroxidase, produces a colored chromagen that is measured at 520nm. The color intensity at 520nm is proportional to the concentration of Uric Acid in the sample.

### Reagents

Uric Acid reagent (concentrations refer to reconstituted reagent). 4-aminoantipyrine >0.3mM, TBHBA >1.0mM, Uricase 150 U/L, Peroxidase 2,500 U/L, buffer, pH 8.1±0.1. Non-reactive stabilizers and fillers.

### Precautions

This reagent is for in vitro diagnostic use only.

### Reagent Preparation

Reconstitute reagent with the volume of water stated on the vial label. Swirl gently to dissolve.

### Reagent Storage

Store reagents at 2-8°C.

Reconstituted reagent is stable for at least 2 days at room temperature and 31 days at 2-8°C.

### Reagent Deterioration

Do not use if:

Moisture has penetrated the vial and caking has occurred.

The reagent blank has an absorbance of 0.400 or greater at 520nm. A slight pink color is normal.

### Specimen Collection and Storage

Nonhemolyzed serum is recommended. Uric Acid in serum is stable for three days at 2-8°C and up to six months when frozen.<sup>8</sup>

### Interferences

Bilirubin and ascorbic acid can result in falsely depressed Uric Acid levels.

Lipemic samples may cause falsely elevated Uric Acid levels.

3. See Young, et al.<sup>9</sup> for other interfering substances.

### Materials Provided

Uric Acid Reagent.

### Materials Required but not Provided

Accurate pipetting devices.

Timer.

Test tubes/rack

Spectrophotometer with ability to read at 520 nm.

Heating Block (37°C).

### Procedure (Automated)

Refer to specific instrument application instructions.

### Procedure (Manual)

Reconstitute reagent according to instructions.

Label test tubes: "Blank", "Standard", "Control", "Unknowns", etc.

Pipette 1.0 ml of working reagent into each tube.

Pre-warm at 37°C for at least five minutes.

Add 0.025 ml (25ul) of sample to respective tubes and mix.

Incubate all tubes at 37°C for five minutes.

After incubation, zero spectrophotometer with blank at 520nm. Read and record absorbances of all test tubes.

To determine results, see "Calculations".

### Procedure Notes

If the spectrophotometer being used requires a final volume greater than 1.0ml for accurate reading, use 0.05ml (50ul) of sample to 2.5ml of reagent. Perform the test as described above.

Samples with values exceeding 25mg/dl should be diluted 1:1 with saline, re-assayed and the results multiplied by two.

Lipemic samples will give falsely elevated results and a serum blank must be run.

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

### Calibration

Use an aqueous Uric Acid standard (5mg/dL) or an appropriate serum calibrator.

### Quality Control

Use control serums with known normal and abnormal Uric Acid levels to monitor the integrity of the reactions.

NOTE: Lipemic controls may give falsely elevated results. Follow step #3 of "Procedure Notes".

### Calculations

A = Absorbance

$A(\text{Unk}) \times \text{Conc. of Std.} = \text{Uric Acid (mg/dL)}$

$A(\text{Std})$

Example: unknown A (Unk) = 0.126, A (std) = 0.100, Conc. of Std = 5 mg/dL.

# Uric Acid Reagent Set

Then:  $0.126 \times 5 = 6.3 \text{ mg/dL}$   
0.100

SI Units (mM/L)

Multiply the result (mg/dL) by 10 to convert dL to L and divide by 168 (the molecular weight of Uric Acid).

$\text{mg/dL} \times 10 = \text{mM/L}$      $\text{mg/dL} \times .0595 = \text{mM/L}$   
168

Example:  $6.3 \text{ mg/dL} \times .0595 = 0.375 \text{ mM/L}$

## Expected Values

2.5-7.7mg/dl<sup>8</sup>

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

## Performance

Linearity: 25 mg/dL

Comparison: Testing with another similar enzymatic Uric Acid procedure yielded a correlation coefficient of .996 with a regression equation of  $y=1.03x-0.34$ .

Precision:

Within Run			Run to Run		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
6.58	0.13	1.9	6.78	0.11	1.6
10.91	0.16	1.3	11.34	0.14	1.2

## References

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