

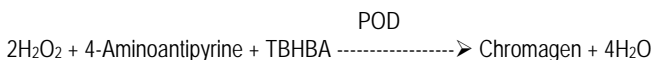
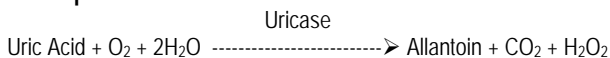
## Intended Use

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, 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

1. Store reagents at 2-8°C.
2. 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:

1. Moisture has penetrated the vial and caking has occurred.
2. 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

1. Bilirubin and ascorbic acid can result in falsely depressed Uric Acid levels.
2. 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

1. Accurate pipetting devices.
2. Timer.
3. Test tubes/rack
4. Spectrophotometer with ability to read at 520 nm.
5. Heating Block (37°C).

## Procedure (Automated)

Refer to specific instrument application instructions.

## Procedure (Manual)

1. Reconstitute reagent according to instructions.
2. Label test tubes: "Blank", "Standard", "Control", "Unknowns", etc.
3. Pipette 1.0 ml of working reagent into each tube.
4. Pre-warm at 37°C for at least five minutes.
5. Add 0.025 ml (25ul) of sample to respective tubes and mix.
6. Incubate all tubes at 37°C for five minutes.
7. After incubation, zero spectrophotometer with blank at 520nm. Read and record absorbances of all test tubes.
8. To determine results, see "Calculations".

## Procedure Notes

1. 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.
2. Samples with values exceeding 25mg/dl should be diluted 1:1 with saline, re-assayed and the results multiplied by two.
3. 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.

# Uric Acid Reagent Set

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

## Calculations

A = Absorbance

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

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

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

## 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).

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

Example: 6.3mg/dL x .0595 = 0.375mM/L

## Expected Values

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

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

## Performance

1. Linearity: 25 mg/dL
2. Comparison: Testing with another similar enzymatic Uric Acid procedure yielded a correlation coefficient of .996 with a regression equation of  $y=1.03x-0.34$ .
3. Precision:

Within Run			Run to Run		
<u>Mean</u>	<u>S.D.</u>	<u>C.V.%</u>	<u>Mean</u>	<u>S.D.</u>	<u>C.V.%</u>
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

1. Folin, D., Dennis, W., J. Biol. Chem. 13:469 (1913).
2. Caraway, W.T., Clin. Chem. 4:239 (1963).
3. Morin, L.G., J. Clin. Path. 60:691 (1973).
4. Morin, L.G., Clin. Chem. 20:51 (1974).
5. Brochner-Mortenson, K., Medicine 19:161 (1940).
6. Klackar, H.M., J. Biol. Chem. 167:429 (1947).
7. Praetorius, E., Poulson, H., Scand. J. Clin. Invest 5:273 (1953).
8. Henry, R.J., Clinical Chemistry: Principles and Technics, 2<sup>nd</sup> Ed., Hagerstown (MD), Harper & Row, pp.531 &541 (1974).
9. Young, D.S., et al. Clin. Chem. 21:1D (1975).

Manufactured by Pointe Scientific, Inc.  
5449 Research Drive, Canton, MI 48188



European Authorized Representative:  
Obelis s.a.

Boulevard Général Wahis 53  
1030 Brussels, BELGIUM

Tel: (32)2.732.59.54 Fax:(32)2.732.60.03 email: mail@obelis.net

Rev. 12/09 P803-U7580-02