POINTE SCIENTIFIC, INC.
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Uric Acid Reagent Set

Intended Use
For the quantitative determination of Uric Acid in serum.

Method History
Uric Acid has been determined by phosphotungstate methods,[1] variations of the phosphotungstate method[2] and iron reduction methods.[3,4] The above methodologies are influenced by many substances in their procedures as well as many contaminating substances on glassware, etc.[5] The enzyme Uricase has been widely used for Uric Acid determinations because of its improved specificity.[6,7] 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 peroxide to yield a chromagen measured at 520nm.

Principle
$$\text{Uric Acid} + O_2 + 2H_2O \xrightarrow{\text{Uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

$$2H_2O_2 + \text{4-Aminoantipyrine} + \text{TBHBA} \xrightarrow{\text{POD}} \text{Chromagen} + 4H_2O$$

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.[8]

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.[9] 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

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

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

Then: 0.126 x 5 = 6.3 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).

mg/dL x 10 = mM/L mg/dL x .0595 = mM/L
168

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

Expected Values

2.5-7.7mg/dl[8]
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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