

Uric Acid Reagent

Catalog #: 43725

for use with the

SDI CA480 Clinical Chemistry System

INTENDED USE

For the *in vitro* quantitative determination of Uric Acid in serum.

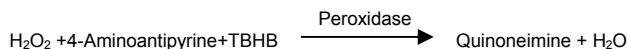
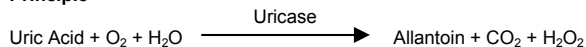
SUMMARY AND EXPLANATION¹⁻³

Uric Acid measurements are most commonly used in the diagnosis of gout. Increased levels (hyperuricaemia) may be observed in leukemia, polycythaemia, atherosclerosis, diabetes, hypothyroidism, and conditions associated with decreased renal function. Decreased levels are present in patients with Wilson's Disease.

METHODOLOGY

Uric Acid has historically been determined by variations of colorimetric phosphotungstate^{4,5} and iron reduction^{6,7} methods. Recent methodologies use the enzymes uricase and hydrogen peroxidase, along with a chromogen to yield a colorimetric end product. These methods demonstrate better specificity for uric acid^{8,9,10}. This procedure uses uricase, peroxidase and the chromogen TBHB to yield a colorimetric end product. The colorimetric end product produced in this reaction can be measured at 520nm and is proportional to the uric acid concentration in the sample.

Principle



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

REAGENT COMPOSITION

Active Ingredients	Concentration
4-Aminoantipyrine	0.5 mM
TBHB	1.75 mM
Uricase (Bacillus Sp.)	>32 U/L
Peroxidase (Horseradish)	>1300 U/L
Non-reactive stabilizers and fillers.	
pH 8.0.	

Precautions:

- This reagent is for *in vitro* diagnostic use only.
- Reagent contains Sodium Azide (0.05%) as a preservative. In a dry state may react with copper or lead plumbing to form explosive metal azides. Upon disposal, flush with large amounts of water to prevent azide build up.

REAGENT PREPARATION

Reagent comes in a ready to use form.

REAGENT STORAGE

- Store the reagent at 2-8°C (refrigerated).
- The reagent is stable until the expiration date when stored at 2-8°C.

REAGENT DETERIORATION

Do not use the reagent if:

- The reagent is turbid.
- The reagent blank has an absorbance of 0.50 or greater at 520nm.
- The working reagent does not meet stated performance parameters.

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.²

INTERFERENCE

Studies to determine the level of interference for hemoglobin, bilirubin, and lipemia were carried out, the following results were obtained:

Hemoglobin:

No significant interference ($\pm 10\%$) from hemoglobin up to 200 mg/dL.

Bilirubin:

No significant interference ($\pm 10\%$) from bilirubin up to 25.0 mg/dL.

Lipemia:

No significant interference ($\pm 10\%$) from lipemia up to 1000 mg/dL measured as triglycerides.

See Young, et al.¹¹ for other interfering substances.

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

- SDI CA480 Clinical Chemistry System
- Deionized water and related equipment, e.g.: pipettes
- Analyzer specific consumables, e.g.: sample cups
- Control, and Calibrator materials such as those provided by SDI Biomed

ASSAY PROCEDURE

System Parameters

Uric Acid (Liquid)

TEMPERATURE:	37°C
WAVELENGTH:	520 nm
ASSAY TYPE:	End Point
DIRECTION:	Increase
SAMPLE / RGT RATIO:	1 : 50
e.g. Sample Vol.	0.003 mL (3μL)
Reagent Vol.	0.150 mL (150 μL)
INCUBATION TIME:	5 min

Procedure Note:

The reagent and sample volumes may be altered proportionally to accommodate various instrument requirements.

Calculations:

(A = Absorbance)

$$\frac{A(\text{patient})}{A(\text{standard})} \times \text{Concentration of standard (mg/dL)} = \text{Uric Acid (mg/dL)}$$

Example:

A (patient) = 0.071

A (standard) = 0.302

Concentration of standard = 12.1 mg/dL.

$$\frac{0.071}{0.302} \times 12.1 = 2.8 \text{ mg/dL Uric Acid}$$

Limitations:

- Samples with values exceeding 25.0 mg/dL should be diluted 1:1 with saline and re-run. The final answer should be multiplied by two.
- The reagent and sample volumes may be altered proportionally to accommodate various instrument requirements.

CALIBRATION

Use an aqueous Uric Acid standard, or an appropriate serum calibrator.

QUALITY CONTROL

The integrity of the reaction should be monitored by use of a two level control with known Uric Acid values.

EXPECTED VALUES¹²

Child:	2.0 – 5.5 mg/dL
Adult Male:	3.5 - 7.2 mg/dL
Adult Female:	2.6 – 6.0 mg/dL

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

PERFORMANCE

Linearity:

When run as recommended the assay is linear from 0.0 to 25.0 mg/dL

Method Comparison:

Studies performed between this procedure and a similar methodology yielded the following results:

Number of samples pairs:	42
Range of samples:	0.4 – 23.0 (mg/dL)
Correlation Coefficient:	0.9987
Slope:	0.9755
Intercept:	0.31 (mg/dL)

Precision:

Within Run	Level 1	Level 2	Level 3
n=40			
Mean (mg/dL)	3.38	6.52	11.09
S.D. (mg/dL)	0.05	0.10	0.09
C.V. (%)	1.6	1.5	0.8

Total

n=40 (10 days / 2 runs per day / 2 replicates per run)

Mean (mg/dL)	3.38	6.52	11.09
S.D. (mg/dL)	0.06	0.12	0.15
C.V. (%)	1.9	1.8	1.4

Sensitivity:

A calibration factor of approximately 119 was obtained, which is equivalent to a sensitivity of 0.0084 D Abs per mg/dL.

REFERENCES

- Searcy RL: Diagnostic Biochemistry. McGraw-Hill, New York, NY, (1969).
- Henry RJ, Common C, Winkelman JW (eds), Clinical Chemistry: Principles and Techniques. Harper & Row, Hagerstown, MD, (1974).
- Balls ME: Adv Clin Chem 18:213, (1976).
- Folin D., Dennis, W., J. Biol. Chem. 13:469 (1913).
- Caraway, W.T., Clin. Chem. 4:239 (1963).
- Morin, L.G., J. Clin. Path. 60:691 (1973).
- Morin, L.G., Clin. Chem. 20:51 (1974).
- Brochner-Mortenson, K., Medicine 19:161 (1940).
- Klackar, H.M., J. Biol. Chem. 167:429 (1947).
- Praetorius, E., Poulton, H., Scand. J. Clin. Invest 5:273 (1953).
- Young, D.S., et al. Clin. Chem. 21:1D (1975).
- Tietz Textbook of Clinical Chemistry, WB Saunders Co., Philadelphia PA, 2nd Ed (1994).

Manufactured for:



23679 Calabasas Road, Unit 241
Calabasas, CA 91302
800-952-2470

PI: 43725.01

Rev. 09/05/06