Uricostat
enzimático AA

For acid uric determination in serum, plasma or urine

SUMMARY
Uric acid is a metabolite found in purines, nucleic acids and nucleoproteins.
Serum uric acid concentration usually varies from one individual to another depending on several factors such as: sex, diet pattern, ethnic origin, genetic constitution, pregnancy. Abnormal levels of serum uric acid indicate metabolic disorders of its precursors or inadequate excretion.

PRINCIPLE
The reaction system is as follows:

$$\text{uric acid} + 2\ H_2O + O_2 \xrightarrow{\text{UOD}} \text{allantoin} + H_2O_2 + CO_2$$

$$2\ H_2O_2 + \text{4-AP} + \text{3,5-SDH} \xrightarrow{\text{POD}} \text{quinoneimine}$$

PROVIDED REAGENTS
S.Standard: 10 mg/dl uric acid solution.
A. Reagent A: vials containing uricase (UOD), peroxidase (POD), 4-amino-phenazone (4-AP) and potassium ferrocyanide.
B. Reagent B: sulfonic dichlorohydroxybenzene (SDH) in phosphates buffer solution pH 7.4.

Final concentrations

UOD	≥ 100 U/l
POD	≥ 600 U/l
4-AP	0.10 mmol/l
Potassium ferrocyanide	6 umol/l
SDH	2.0 mmol/l

NON-PROVIDED REAGENTS
Wiener lab.'s **Calibrador A plus**.

INSTRUCTIONS FOR USE
Standard: ready to use.
Working Reagent: dissolve a vial containing Reagent A in a Reagent B bottle. Rinse the vial several times with Reagent B. Mix until complete dissolution. Homogenize and date.

WARNINGS
Reagents are for “in vitro” diagnostic use.
Use the reagents according to the working procedures for clinical laboratories.
The reagents and samples should be discarded according to the local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS
Provided Reagents: stable in refrigerator (2-10ºC) until the expiration date shown on the box. Do not expose to high temperatures for long periods.
Working Reagent: stable in refrigerator (2-10ºC) for 30 days from preparation date.

INSTABILITY OR DETERIORATION OF REAGENTS
During use, the Working reagent may develop a slight pink color, which does not affect the performance, provided that a Blank is run with each batch of tests and a Standard periodically.
Discard every time Blank readings are above 0.160 O.D. or when Standard readings are abnormally low.

SAMPLE
Serum, plasma or urine
a) Collection: obtain serum or plasma as usual. Remove serum from clot as soon as possible within two hours from collection. If urine is used, it should be preferably fresh.
b) Known interfering substances:
- Drugs: strongly reducing substances, such as ascorbic acid (Vitamin C), Hyoscine butyl bromide, etc. administered at high doses interfere. Therefore, therapy should be discontinued 24 hours before sample collection whenever possible.
- No interference was observed from: bilirubin up to 120 mg/l, triglycerides up to 840 mg/dl, nor hemoglobin up to 180 mg/dl.

See Young, D.S. in References for effect of drugs on the present method.
c) Stability and storage instructions: samples should be preferably fresh. If assay cannot be immediately performed, serum or plasma samples may be stored for up to 3 days at 20-25ºC, 7 days at 2-10ºC or 6 months at -20ºC without preservatives. Urine samples may be stored at pH > 8 for up to 4 days at 20-25ºC. Do not refrigerate or freeze.

REQUIRED MATERIAL (non-provided)
- Spectrophotometer or photocolorimeter.
- Adequate volumetric material.
- Tubes or spectrophotometric square cuvettes.
- Water bath at 37ºC.
- Watch or timer.

ASSAY CONDITIONS
- Wavelength: 505 nm in spectrophotometer or in photocolorimeter with green filter (490-530 nm)
- Reaction temperature: 37ºC or room temperature (18-25ºC)
- Reaction time: 5 minutes (at 37ºC) or 20 minutes at room temperature

- Sample volume: 20 ul
- Working Reagent volume: 1 ml
- Final reaction volume: 1.02 ml

Sample and Reagent volumes may be proportionally decreased or increased (e.g. 50 ul Sample + 2.5 ml Working Reagent or 100 ul + 5 ml)

PROCEDURE

In three test tubes or spectrophotometer cuvettes labeled B (Blank), S (Standard) and U (Unknown), place:

	B	S	U
Standard	-	20 ul	-
Sample	-	-	20 ul
Working Reagent	1 ml	1 ml	1 ml

Mix gently and incubate in water bath at 37°C for 5 minutes or at room temperature (18-25°C) for 20 minutes. Remove from bath. Let cool. Read in colorimeter with green filter (490-530 nm) or in spectrophotometer (505 nm), setting instrument to zero O.D. with the Blank.

URINE TECHNIQUE

Follow the above technique diluting the sample 1/10 with water or saline. Calculate the results, multiplying by the dilution factor used.

STABILITY OF FINAL REACTION

Final reaction color is stable for 30 minutes, thus absorbance should be read within that period.

CALCULATIONS

$$\text{uric acid (mg/dl)} = U \times f \qquad \text{where } f = \frac{10 \text{ mg/dl}}{S}$$

QUALITY CONTROL METHOD

Each time the test is performed, analyze two levels of a quality control material (**Standatrol S-E 2 niveles**) with known uric acid concentration.

REFERENCE VALUES

The following range is reported in normal adults with standard protein intake:

Men: 2.5-6.0 mg/dl
Women: 2.0-5.0 mg/dl

In the literature (Tietz, N.W.) the following reference value range is mentioned:

Serum or plasma

Men: 3.5-7.2 mg/dl
Women: 2.6-6.0 mg/dl

Urine

250 a 750 mg/24 hours

It is recommended that each laboratory establishes its own intervals and reference values, taking into consideration age, sex, dietary habits and other factors.

SI SYSTEM UNITS CONVERSION

Uric acid (mg/dl) x 0.059 = Uric acid (mmol/l)
Uric acid (mg/24 hs) x 0.0059 = Uric acid (mmol/24 hs)

PROCEDURE LIMITATIONS

See Known interfering substances under SAMPLE.
Other causes of erroneous results are:
Contamination: reducing agents decrease color response, whereas oxidants color the Reagent increasing the Blanks. Detergents, heavy metals and cyanides inhibit enzymes.

PERFORMANCE

The assays were performed in an Express plus analyzer (*).
a) Reproducibility: the following results were obtained:

Intra-assay precision (n = 20)

Level	S.D.	C.V.
5.4 mg/dl	± 0.094 mg/dl	1.75 %
9.6 mg/dl	± 0.170 mg/dl	1.78 %

Inter-assay precision (n = 30)

Level	S.D.	C.V.
5.61 mg/dl	± 0.146 mg/dl	2.61 %
9.69 mg/dl	± 0.232 mg/dl	2.39 %

b) Recovery: by adding known amounts of uric acid to different sera, a recovery between 97 and 101% was obtained for 10 mg/dl uric acid level.
c) Sensitivity studies: minimum detection limit is 0.040 mg/dl and analytical sensitivity is 0.473 mg/dl.
d) Linearity: reaction is linear up to 20 mg/dl. For higher values, repeat determination using half sample volume and multiply final result by 2.
e) Correlation: uric acid values of 107 specimens were determined using Wiener lab.'s **Uricostat enzimático AA** kit and a commercial kit based on the same principle. The correlation coefficient was:
r = 0.9961, slope b = 1.0321 and intercept a= - 0.0427

PARAMETERS FOR AUTOANALYZERS

For programming instructions check the user manual of he autoanalyzer in use.
For calibration, it can be used Wiener lab.' **Calibrador A plus**.

WIENER LAB. PROVIDES

- 2 x 50 ml (Cat. Nº: 1840106).
- 4 x 50 ml (Cat. Nº: 1840105).

REFERENCES

- Chu, S.Y. - Can. J. Med. Technol. 40/5:154 (1978).
- Day, J.H. - La Prensa Médica Argentina Vol. 58 Nº 15:786 (1971).
- Donadon, V.; Barbieri, E.; Menin, A.; Canterin, A. - LAB Vol. III Nº 4:473 (1976).
- International Federation of Clinical Chemistry - Clin. Chim. Acta 87/3:459 F (1978).
- Trinder, P. - Ann. Clin. Biochem. 6/24 (1969).
- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 4th ed., 2001.

(*) TM Ciba Corning Diagnostics

- Henry, R. J. - Clinical Chemistry, Principles and Technics; Harper & Row, publisher; 1964.
- Tietz Fundamentals of clinical chemistry - Burtis, C., Ashwood, E. (5th Edition) WB Saunders, 2001.

SYMBOLS

The following symbols are used in the packaging for Wiener lab. diagnostic reagents kits.


This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices


Authorized representative in the European Community


"In vitro" diagnostic medical device


Contains sufficient for <n> tests


Use by


Temperature limitation (store at)


Do not freeze


Biological risks

Volume after reconstitution


Contents


Batch code


Manufactured by:


Harmful


Corrosive / Caustic


Irritant


Consult instructions for use


Calibrator


Control


Positive Control


Negative Control


Catalog number

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