STANBIO LABORATORY
An EKF Diagnostics Company

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Stanbio Uric Acid LiquiColor®
Procedure No. 1045

Quantitative Enzymatic Colorimetric Determination of Uric Acid in Serum, Plasma or Urine

Summary and Principle[1,2]

Uricase acts upon uric acid to form hydrogen peroxide and allantoin. The H_2O_2 is measured quantitatively by its reaction with 3,5-dichloro-2-hydroxybenzenesulfonic acid (DCHB), in the presence of peroxidase and 4-aminophenazone, to form a red violet quinoneimine complex. Lipid Clearing Factor (**LCF**): a mixture of special additives developed by Stanbio is intregrated into the uric acid reagent to help minimize interference due to lipemia.

1. Uric Acid + H_2O + $\xrightarrow{\text{Uricase}}$ H_2O_2 + CO_2 + Allantoin

2. H_2O_2 + DCHB + 4-aminophenazone $\xrightarrow{\text{Peroxidase}}$ N-(4-antipyryl)-3-chloro-5-sulfonate-p-benzo-quinonemonoimine + H_2O

Reagents

Enzymatic Uric Acid Reagent (Liquid), Ref. No. 1046

Phosphate buffer, pH 7.0	50 mmol/L
3,5-Dichloro-2-hydroxybenzenesulfonic acid	4 mmol/L
4-Aminophenazone	0.3 mmol/L
Peroxidase	> 1000 U/L
Uricase	> 200 U/L

Stabilizers and activators in a buffered solution.

Enzymatic Uric Acid Standard, 8 mg/dL, Ref. No. 1044

Aqueous solution of uric acid with solubilizer and stabilizer added.

Precautions: *For In Vitro Diagnostic Use.* Dispose of reagents in accordance with local requirements.

Reagent Preparation: Reagent and standard are ready for use.

Reagent Storage and Stability: Reagent is stable, stored at 2-8°C, until expiration date on label. Once opened, contamination must be avoided. Standard is stable until expiration date on label when stored at 2-8°C. DO NOT FREEZE. Bring reagent to room temperature before use.

Note: To prevent contamination of enzymatic reagent, pour into a separate vessel a volume slightly in excess of that required. Do not return unused portion to bottle.

Materials Required But Not Provided

Spectrophotometer, capable of absorbance readings at 520 nm.
Cuvets, 10 or 12 x 75 mm
Heat block/bath, 37°C (optional)
Accurate pipetting devices

Specimen Collection and Preparation

Serum, or plasma from heparinized or EDTA blood, is recommended. Urine is diluted 1:10 (1 + 9) with distilled water.

Sample Stability: Uric acid in serum, plasma and urine is reported stable for 2-3 days at room temperature, 3-7 days at 2-8°C and for 6-12 months when frozen.

Interfering Substances: Hemoglobin levels greater than 100 mg/dL, and bilirubin greater than 20mg/dL, will affect results. Refer to Young et al.[5] for a listing of other interfering substances.

Automated Analyzer

Parameters:

Wavelength	520 nm
Reaction Type	Endpoint
Reaction Direction	Increasing
Reaction Temperature	37°C
Sample/Reagent Ratio	1:50
Equilibration Time	3 Seconds
Read Time	4 Seconds
Lag Time	300 seconds
Blank Absorbance Limit	0.090A
High Absorbance	0.700A
Standard	8.0 mg/dL
Low Normal	2.4 mg/dL
High Normal	7.0 mg/dL
Linearity	20.0 mg/dL

Manual Procedure

1. Pipet into cuvets the following volumes (ml) and mix well:

	Reagent Blank (RB)	Standard (S)	Sample (U)
Reagent	1.0	1.0	1.0
Standard	—	0.02	—
Sample	—	—	0.02

Note: For instruments requiring volumes greater than 1.0 mL, use 2.0 mL reagent and 0.05 mL standard and sample.

2. Incubate all cuvets at 37°C for 5 minutes and allow to cool, or incubate at room temperature for 15 minutes.

3. Read S and U vs. RB at 520 nm within 15 minutes.

Quality Control: Two levels of control material with known Uric Acid levels determined by this method should be analyzed each day of testing.

Results

Values are derived by the following equations: $\frac{Au}{As}$

$$\text{Serum or Plasma Uric Acid (mg/dL)} = \frac{Au}{As} \times 8$$

$$\text{Urine Uric Acid (mg/dL)} = \frac{Au}{As} \times 80$$

where Au and As are the absorbance values of UNKNOWN and STANDARD, respectively, 8 is the concentration of the standard (mg/dL) and the factor of 80 combines the same standard concentration with the required urine dilution factor of 10.

Expected Values[3,4]

Normal Range:
Men 3.4 – 7.0 mg/dL
Women 2.4 – 5.7 mg/dL

Urine: 0.5 – 1.0 gram/day
(dependent on diet contents of purines)

Performance Characteristics[6]

Reproducibility: A study was performed on a normal control serum (mean = 4.5 mg/dL) and on an abnormal control (mean = 8.6 mg/dL), which entailed a series of 5 assays on each of 5 days. Coefficients of variation (CV) were within run 2.8% and 1.6% and between runs 3.4% and 3.8%, respectively.

Correlation: Determination of uric acid by the procedure described (y) and by the acetaldehyde dehydrogenase-UV reference method (x) on 49 sera (range 2.5-11.4 mg/dL) showed a correlation coefficient (r) of .982 and a regression equation of $y = 1.001x + 0.4$.

Linearity: When performed as directed the method is linear from 0 to 20 mg/dL.

References

1. Barham D. Trinder p: analyst 97:142, 1972.
2. Fossati P et al: Clin Chem 26:227, 1980.
3. Thefeld L et al: Dtsch med Wschr 98:380, 1973.
4. Haisman P, Muller BR: Glossary of Clinical Chemistry Terms. Butterworth, London, 1977, p126.
5. Young DS et al. Clin Chem 21:246D, 1975 (Special Issue).
6. Stanbio Laboratory data.

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