URIC ACID

(LIQUID)

5 x 30 ml

RE – ORDER URA1500

INTENDED USE

This reagent is intended for the quantitative determination of Uric Acid in serum.

METHOD AND HISTORY

Uric Acid has been determined by phosphotungstate methods, variations of the phosphotungstate method and iron reduction methods. The above methodologies are influenced by many substances in their procedures as well as many contaminating substances on glassware, etc. The enzyme Uricase has been widely used for Uric Acid determinations because of its improved specificity. Recently, hydrogen peroxide, a by-product of the Uricase-Uric Acid reaction has been coupled to other enzymatic reactions to yield a colorimetric and product. The present procedure uses the coupling of 4-aminoantipyrine (4-AAP), 2-Hydroxy-3-5 Dichloro-benzenesulfonate (HDCBS), and hydrogen peroxide in the presence of peroxidase to yield a chromagen measured at 520nm.

TEST PRINCIPLE

The hydrogen peroxide formed by the action of uricase on uric acid reacts with DHBS and 4-aminoantipyrine in the presence of peroxidase to form a red colored quinoneimine dye.

Uricase

Uric acid + H_2O + O_2 ----------> Allantoin + CO_2 + H_2O_2

Peroxidase

DHBS + 4-aminoantipyrine + $2H_2O_2$ ----------> Quinoneimine + $4H_2O$

Uric Acid is oxidized by Uricase to allantoin and hydrogen peroxide. HDCBS + 4-AAP + hydrogen peroxide, in the presence of peroxidase, produces a red chromogen that is measured at 520nm. The absorbance at 520nm is proportional to the concentration of Uric Acid in the sample.

CLINICAL SIGNIFICANCE

The determination of uric acid in serum is most commonly performed for the diagnosis of gout. Increased uric acid levels are also found in leukemia, polycythemia, familial idiopathic hyperuricemia, and conditons associated with decreased renal function.

PATIENT PREPARATION

No special patient preparation is required.

SPECIMEN COLLECTION.

Fresh, clear unhemolyzed serum is the preferred specimen. Use a standard venipuncture tube to draw patient sample. The amount of sample required will depend on the analyzer used. The amount of serum required is in the range of 5-25 µl. Call King's technical service department at 1-800-262-8655 for the recommended sample volume for your analyzer. Record the patient's name, date and time of sample collection and preparation.

SPECIMEN STORAGE

Serum uric acid is stable for 3 days at 18°-26°C, 14 days at 2°-8°C, and for 6 months at -20°C . Frozen samples should be thawed at room temperature and mixed completely before analysis. Thawed samples should not be refrozen. It is recommended that testing be done as soon as possible after sample collection and preparation. If testing cannot occur immediately, store the sample properly using the guidelines above.

REAGENT

Uric Acid reagent contains:

peroxidase	> 2,500 U/L
uricase	> 150 U/L
DHBS	2.0 mM
4-aminoantipyrine	0.2mM
sodium azide	0.02%

buffer, preservatives, and stabilizers

WARNINGS AND PRECAUTIONS

For In Vitro Diagnostic Use. Not for Internal use in Humans or Animals. In Vitro Diagnostics reagents may be hazardous. Avoid ingestion and skin or eye contact. This reagent contains sodium azide (0.02%) as a preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large amounts of water.

REAGENT PREPARATION

The reagents are ready to use as is.

REAGENT STORAGE AND STABILITY

All reagents included in the kit are stable at 2-8° C (refrigerated) until the expiration date stated on the labels.

ADDITIONAL MATERIALS REQUIRED

Spectrophotometer or colorimeter capable of reading absorbance at 520 nm.

1 cm cuvettes or a flow cell capable of transmitting light at 520 nm.

Test tubes and pipettes.

Deionized or distilled water for a reagent blank.

Timer .

Calibrator

Normal and abnormal control for quality control.

TEST PROCEDURE

the following is a general procedure for use on a manual instrument. Application procedures for use on an automated analyzers are available.

PROCEDURE CONDITIONS

Wavelength	520 nm
Temperature	37° C
Pathlength	1.0 cm
Mode	endpoint
Incubation time	5 min
Sample to reagent ratio	1:40

INSTRUMENT

Any instrument capable of reading absorbance accurately with a sensitivity of 0.001 absorbance at 520 nm may be used. The band width should be 10 nm or less, stray light 0.5% or less, and the wavelength accuracy within 2 nm.

CALIBRATION

Use serum based calibrator. The uric acid assay is calibrated by referencing the absorbance of the unknown sample to the absorbance of the calibrator.

PROCEDURE

Prepare the required volume of working reagent. (See Reagent Preparation section.) Into separate test tubes pipette 25 µl of distilled water, calibrator, or serum to be assayed. Add 1.0 ml of working reagent and mix.

Incubate for 5 minutes at 37° C and determine the absorbance of the calibrator (As) and of each serum (A) at 520 nm using the distilled water sample as the reagent blank.

PROCEDURE NOTE

The color of the final reaction mixture is stable for 30 minutes.

CALCULATION AND RESULTS

$$\text{Uric acid (mg/dl)} = \frac{A}{As} \times \text{concentration of calibrator}$$

A = absorbance of sample,

As = absorbance of calibrator

$$\text{Example: Uric acid (mg/dl)} = \frac{.190}{.270} \times 6.0 = 4.2 \text{ mg/dl}$$

with A = .190 and As = .270 concentration of calibrator = 6.0 mg/dl

EXPECTED VALUES

2.5 - 7.7mg/dl

It is strongly recommended that each laboratory establish its own normal Range

MEDICAL ALERT VALUES

Each laboratory should establish low and high values beyond which the patient would require immediate attention by a physician. If a "medical alert value" is reached, always repeat the test to confirm the result and notify a physician if the result is confirmed.

LIMITATIONS OF PROCEDURE

14752 FRANKLIN AVE., SUITE D, TUSTIN, CA 92780 · USA
714-734-8041 / 714-734-8036 [FAX]

Elevated values of bilirubin and ascorbic acid will cause falsely decreased uric acid concentrations. Grossly lipemic or turbid samples can result in falsely elevated uric acid concentrations. Young gives a list of drugs and other substances that interfere with uric acid levels.

QUALITY CONTROL

Standard practice for quality control should be applied to this system. Commercially available lyophilized controls can be used to monitor the daily acceptable variations. Normal and abnormal controls should be assayed at the beginning of each run of patient samples, whenever a new reagent or a different lot number is being used, and following any system maintenance. A satisfactory level of performance is achieved when the analyte values obtained are within the "acceptable range" established by the laboratory.

CALIBRATION PROCEDURES

Use serum based calibrator kit. The uric acid assay is calibrated by referencing the absorbance of the unknown sample to the absorbance of the calibrator. Refer to your instrument manual for more details.

Calibration is required with the use of a new lot of reagent, any system maintenance or whenever indicated by quality control data.

PRECISION

The estimates of precision shown below were obtained from assays of human control serum.

Within-Run

Mean (mg/dl)	SD (mg/dl)	CV (%)
6.0	0.06	1.0
6.9	0.04	0.6
9.9	0.06	0.6

Between-Run

Mean (mg/dl)	SD (mg/dl)	CV (%)
6.1	0.16	2.6
7.2	0.16	2.2
10.2	0.31	3.0

CORRELATION

Correlation: A correlation study was done using this method and a comparative Uric Acid method. The samples range between 1.9 mg/dl and 10.5 mg/dl.

Number of Samples	Regression Equation y=King, x=comparative	Correlation Coefficient
132	y = 0.998 x - .017	0.999

LINEARITY

This procedure is linear through 20 mg/dl beyond which the specimen should be diluted 1 to 1 with deionized water. Reassay the specimen and multiply the results by 2.

REFERENCES

Folin, D., Dennis, W., J. Biol. Chem. 13:469(1913).
Caraway, W.T., Clin. Chem. 4:2239(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., Poulson, H., Scand. J. Clin. Invest. 5:273(1953).
NCCLS document "Protection of Labortory Workers from Infectious Disease Transmitted by Blood, Body Fluids, and Tissue", 2nd Ed.(1991).
Henry, R.J., Clinical Chemistry: Principles and Technics, 2nd Ed., Hagerstown (MD), Harper & Row, pp.531 & 541(1974).
NCCLS document "Procedures for the Collection of Diagnostic Blood Specimens by Skin Puncture:, 3rd Ed.(1991).
Young, D.S., et al Clin. Chem. 21:1D(1975)NCCLS document "Evaluation of Precision Performance of Clinical Chemistry Devices", 2nd Ed. (1992).
Tietz, N.W., (Editor) Fundamentals of Clinical Chemistry, 2nd Ed. (1982) W. B. Saunders Co. Toronto.

Manufactured For:
ClearChem Diagnostics