CATACHEM

# *URIC ACID*
# *MANUAL/AUTOMATED APPLICATIONS*
# *C134-01, V134-12*

**Intended Use**
For **IN VITRO quantitative** determination of Uric Acid in serum.

**Clinical Significance**
Determinations of Uric Acid are primarily used for diagnosing leukemia, polycythemia, gout, renal failure, psoriasis, as well as, for monitoring the causes and treatments. (1)

**Method History**
Automated methods with uricase: ureate oxidase (EC1.7.3.3) are discussed by Gochman and Schmitz. (2) The first Uric Acid method utilizing immobilized uricase for automated systems was introduced by L.P. Leon, et al. (3) These authors reported the superiority of the immobilized uricase for its long-term stability at room temperature and the capability of assaying several thousands of samples with the same enzyme unit. The Catachem Uric Acid method for manual or automated chemistry is a modification of the method reported by L.P. Leon. (4)

**Method Principle**
The uricase enzyme catalyzes the oxidation of Uric Acid to produce allantoin and hydrogen peroxide. The hydrogen peroxide formed is quantitated by oxidative coupling of 4-aminoantipyrine with 3-5 dichloro-2-hydroxybenzene sulfonic acid (DHBS) in the presence of peroxidase. (5) The intensity of the color produced is directly proportional to the concentration of the Uric Acid in the sample. The color complex formed is read at ~ 500nm. The reaction scheme illustrates the reactions that occur in this method.

$$UA + 0_2 + 2H_20 \xrightarrow{\text{Uricase}} \text{Allantoin} + C0_2 + H_20_2$$

$$H_20_2 + \text{DHBS} + 4\text{AA} \xrightarrow{\text{Peroxidase}} \text{Quinonemine Dye}$$

**Reagent Content**
The concentrations of the active ingredients in the reagents will be approximately as follows:

Each liter contains:

| | |
|---|---|
| DHBS | 4 mmol |
| 4-aminoantipyrine | 1.0 mmol |
| Peroxidase (horseradish) | $\geq$ 9000 U |
| Uricase (microbial) | $\geq$ 400 U |
| Buffer | |
| Nonreactive ingredients and stabilizers | |

**Precautions**
Avoid contact of reagents with skin and eyes. Should contact occur, wash affected area with plenty of cold water. **DO NOT PIPETTE BY MOUTH.**

**Preparation Of Working Reagent**
Catachem Uric Acid Reagent is ready to use.

**Reagent Storage And Stability**
Store the Catachem Uric Acid reagent at 2-8˚C. When stored as directed, this reagent is stable until the expiration date stated on the label. Once opened the reagent is stable for 30 days when stored at 2-8˚C and capped when not in use.

**Specimen Collection And Preparation**
The use of clear, unhemolyzed serum that has been separated from the clot as soon after collection as possible is recommended. Uric Acid in serum is stable for several days at room temperature and for longer periods of time when stored at 2-8˚C. (1)

**Interfering Substances**
A comprehensive discussion has been reported on the effects of interfering substances on a uricase assay of Uric Acid in serum (4). A summary of the influence of drugs on clinical laboratory procedures may be found by consulting D.S. Young, et al. (13)

**Expected Values**
The range of expected values determined for this method is 2.2 - 7.7 mg/dL for adult females and 3.7 - 9.0 mg/dL for adult males. These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the species under test and for the area in which the laboratory is located.

**Procedure**
**Important:** Read entire procedure instructions before proceeding with assay.

**Materials Provided (Not Provided)**

| | |
|---|---|
| Spectrophotometer | |
| Match cuvettes | 1 cm light path |
| Timer | to time incubation time |
| Pipette | 1.0 ml for reagent |
| Pipette | 0.02 ml for sample |
| Cylinder | 25ml for preparing reagent |

Constant temp incubator for assays at 37˚C.

**Materials Provided**
Uric Acid Reagent

**Analytical Parameters**

| | |
|---|---|
| Wavelength | 500nm |
| Temperature | 37˚C |
| Pathlength | 1 cm |
| Reaction mode | endpoint |
| Reaction time | 5 minutes 37˚C |
| Reagent volume | 1.0 ml |
| Sample volume | 0.02 ml (20 µL) |
| Total volume | 1.02 ml |
| Sample-to-reagent ratio | 1:51 |

**Assay Procedure**
1. Pipette 1.0 ml of Reagent into each of three cuvettes marked “Calibrator”, “Sample”, and “Blank”.
2. Pipette 0.05 ml of Calibrator, Sample, and water into the cuvettes marked “Calibrator”, “Sample”, and “Blank” respectively.
3. Incubate all cuvettes for 5 minutes at 37˚C.
4. Set spectrophotometer wavelength at 500 nm and zero the instrument with the cuvette marked “Blank”.
5. Read the absorbance of both “Calibrator” and “Sample”.
6. Calculate the Uric Acid concentration (mg/dL) in the sample(s), as shown in calculations and results.

**Results and Calculations**

$$\text{UA (mg/dL)} = \frac{\text{Sample Absorbance}}{\text{Calibrator Absorbance}} \times \text{Calibrator (mg/dL)}$$

Example:

| | | |
|---|---|---|
| Sample Absorbance | = | 0.100 |
| Calibrator Absorbance | = | 0.050 |
| Calibrator (mg/dL) | = | 5 |
| Uric Acid (mg/dL) | = | $\frac{0.100}{0.050} \times 5$ |
| | = | 10 mg/dL |

**Quality Control**
To monitor the performance of the Working Reagent and the procedure used, we recommend the regular use of a normal and abnormal control serum.

**Method Performance Characteristics**
**Sensitivity:** 0.014 - 0.018 absorbance units per mg/dL.
**Linear Range:** 0-20 mg/dL.
**Precision:** Within-run and day-to-day precision is summarized below.

**Uric Acid Precision Study**

| Uric Acid | Within-Run | | Total Precision | |
|---|---|---|---|---|
| Mean | SD | CV | SD | CV |
| mg/dL | mg/dL | % | Mg/dL | % |
| 2.1 | 0.0 | 0.0 | 0.05 | 2.2 |
| 11.6 | 0.08 | 0.65 | 0.08 | 0.68 |
| 21.5 | 0.09 | 0.42 | 0.12 | 0.54 |

**Correlation**
A comparison of this method using an automated analyzer as a reference Uric Acid method based upon the uricase peroxidase reaction resulted in the following regression statistics:

| | | |
|---|---|---|
| Range | = | 2.5 - 15.1 |
| N | = | 124 |
| Y | = | 0.991x - 0.198 |
| r | = | 0.997 |
| Sy.x | = | 0.23 |

**References**

1. Fundamentals of Clinical Chemistry. Edited by Norbert Tietz. WB Saunders, Philadelphia (1976).
2. Gochman N, Smitz JM. Clin Chem 17 (1971) 1154.
3. Leon LP, Smith JB, Snyder LR, Horvath C. Clin Chem 24 (6):1023 (1978).
4. Leon LP, Smith JB, Yeung A, Yeh CK, Horvath C. Journal of Automatic Chemistry 4 No 1 (1982).
5. Trinder P. Ann Clin Biochem 6, 24 (1969).
6. Young DS, Pestaner LD, Gibberman V. Clin Chem 21 No 5 (1975).


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