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# URIC ACID (LIQUID) REAGENT SET

## INTENDED USE
For the quantitative determination of uric acid in serum.

## INTRODUCTION
Uric acid is the end product of purine metabolism. Nearly half of the total uric acid is eliminated and replaced each day by way of urinary excretion and through microbial degradation in the intestinal tract. Increased uric acid levels are commonly associated with both nitrogen retention and urea, creatine, and other non-protein constituents. The quantitation of uric acid is an aid in the diagnosis of gout, decreased renal function, myeloproliferative disorders, and other conditions in which the cause for the hyper- uricemia is not well known.[1]

Uric acid is most commonly determined by a phosphotungstate method[2] and iron reduction method.[3] Due to serum interferences, the enzyme uricase has been widely used instead. Uricase is more specific for uric acid since uricase acts only on uric acid.[4,5]

## PRINCIPLE
The enzymatic reaction sequence employed in the assay of uric acid is as follows:

$$\text{Uric acid} + O_2 + 2\,H_2O \xrightarrow{\text{Uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

$$2\,H_2O_2 + \text{4-Aminoantipyrine} + \text{DHBS} \xrightarrow{\text{Peroxidase}} \text{chromogen} + 4\,H_2O$$

Uric Acid is converted by uricase into allantoin and hydrogen peroxides. The hydrogen peroxide initiates the coupling of 4-aminoantipyrine to 3,5-dichloro-2-hydroxybenzene sulfonic acid (DHBS) to form the chromogen which is measured at 520nm and which is proportional to the amount of hydrogen peroxide generated from uric acid.

## REAGENTS
1. Uric acid reagent: 4-Aminoantipyrine 4mM, 3,5 Dichloro-2- hydroxybenzenesulfonate 2mM, Stabilizer and Surfactant, buffer pH 7.5.
2. Uric acid standard (5 mg/dl).

## WARNINGS AND PRECAUTIONS
1. For *in vitro* diagnostic use.
   *CAUTION*: The reagents may be hazardous. Handle in accordance with good laboratory procedures, which dictate avoiding ingestion, and eye or skin contact.
2. Serum specimens should be considered infectious and handled appropriately.

## STORAGE AND STABILITY
The reagent set is stored refrigerated (2 - 8°C). DO NOT FREEZE. Bring reagent to room temperature before use.

## REAGENT DETERIORATION
The reagent should be discarded if:
1. Turbidity has occurred; turbidity may be a sign of contamination.
2. There is evidence of discoloration. A slight pink color is normal.

## SPECIMEN COLLECTION
1. Test specimen should be serum and free from hemolysis.
2. Bacterial contamination should be avoided to preserve the loss of uric acid.
3. Uric acid in serum is stable for three (3) days at 2 - 8°C and up to six (6) months when frozen.[6]

## INTERFERING SUBSTANCES
1. Bilirubin and ascorbic acid can result in falsely depressed uric acid levels.
2. Lipemic samples may cause falsely elevated uric acid levels.
3. Collection tubes containing formaldehyde as a preservative must be avoided.
4. For a comprehensive review of drug interferences refer to Young et a1.[7]

## MATERIALS REQUIRED BUT NOT PROVIDED
1. Pipette devices
2. Test tubes/rack
3. Timing device
4. Heating block (37° C)
5. Spectrophotometer capable of reading at 520 nm

## GENERAL INSTRUCTIONS
The reagent for uric acid is intended for use either as an automated procedure on chemistry instruments or as a manual procedure on a suitable spectrophotometer.

## AUTOMATED PROCEDURE
Refer to appropriate application manual available.

## MANUAL PROCEDURE
1. Label test tubes, "reagent blank", "standard", "control", "unknown", etc.
2. Pipette 1.0 ml of working reagent into all tubes.
3. Pre-warm all tubes at 37°C for three (3) minutes.
4. Add 0.025 ml (25 µl) of sample to respective tubes and mix.
5. Incubate all tubes at 37°C for ten (10) minutes.

6. After incubation, zero the spectrophotometer with the reagent blank at 520 nm and read/record the absorbance of all tubes. (Wavelength range: 500 – 550 nm).
7. Repeat procedure for each sample.

** TC MULTI-PURPOSE CALIBRATOR MAY BE USED TO REPLACE STANDARD*

**ALTERNATE VOLUMES**

If the spectrophotometer being used requires a final volume greater than 1.0 ml for accurate reading, use 0.05 ml (50 µl) of sample to 3.0 ml of Reagent. Perform the test as described above.

**PROCEDURAL LIMITATIONS**

The reagent is linear to 25 mg/dl uric acid. Samples with values exceeding 25 mg/dl should be diluted 1:1 with saline, reassayed and the results multiplied by two (2). Lipemic samples will give falsely elevated results and a serum blank must be run.

Serum blank: Add 0.025 ml (25 µl) of sample to 1.0 ml water. Zero the spectrophotometer with water. Read and record absorbance and subtract reading from test absorbance.

**CALCULATIONS (RATIOMETRIC)**

A = Absorbance

$$\frac{A\ unknown}{A\ standard} \times \text{concentration of standard} = \text{value for unknown (mg/dl)}$$

*Example*: If the unknown A = 0.170, standard A = 0.180, concentration standard = 5 mg/dl, then:

$$\frac{0.170}{0.180} \times 5 = 4.7\ \text{mg/dl}$$

SI UNITS (mmol/L): Multiply the result (mg/dl) by 10 to convert dl to liter and divide by 168 (the molecular weight of uric acid).

$$\text{mg/dl} \times \frac{10}{168} = \text{mmol/L} \qquad \text{mg/dl} \times 0.0595 = \text{mmol/L}$$

**QUALITY CONTROL**

It is recommended that controls be included in each set of assays. Commercially available control material with established uric acid values may be used for quality control. The assigned value of the control material must be confirmed by the chosen application. Failure to obtain the proper range of values in the assay of control material may indicate either reagent deterioration, instrument malfunction, or procedural errors.

**EXPECTED VALUES**

1.5 - 7.0 mg/dl[8]

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

**PERFORMANCE CHARACTERISTICS**

1. Linearity: 25 mg/dl
2. Sensitivity: Based on an instrument resolution of 0.001 absorbance, the present procedure has a sensitivity of 0.03 mg/d1.
3. Comparison: A comparison with another commercial enzymatic uric acid procedure yielded a correlation coefficient of 1.00 with a regression equation of y = 1.02 x - 0.22.
4. Precision studies:

**Within Run**

| Mean (mg/dl) | S.D. | C.V. |
|---|---|---|
| 3.9 | 0.06 | 2.0% |
| 7.9 | 0.04 | 1.0% |

**Run-to-Run**

| Mean (mg/dl) | S.D. | C.V. |
|---|---|---|
| 3.9 | 0.08 | 2% |
| 8.4 | 0.50 | 6% |

**REFERENCES**


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