POINTE SCIENTIFIC, INC.
5449 Research Drive • Canton MI 48188 • USA

Uric Acid (Liquid) Reagent Set

Intended Use
For the quantitative determination of Uric Acid in serum. For in vitro diagnostic use only.

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 conditions associated with decreased renal function.

Test Summary
Uric Acid has been determined by phosphotungstate methods,[1] variations of the phosphotungstate method[2] and iron reduction methods.[3,4] The above methodologies are influenced by many substances in their procedures as well as many contaminating substances on glassware, etc.[5] The enzyme Uricase has been widely used for Uric Acid determinations because of its improved specificity.[6,7] Recently, hydrogen peroxide, a by-product of the Uricase-Uric Acid reaction, has been coupled to other enzymatic reactions to yield a colorimetric end 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.

Principle
$$\text{Uric Acid} + O_2 + 2H_2O \xrightarrow{\text{Uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

$$2H_2O_2 + \text{4-AAP} + \text{HDCBS} \xrightarrow{\text{POD}} \text{Chromagen} + 4H_2O$$

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

Reagent Composition
Uric Acid reagent: 4-AAP >0.2mM, HDCBS 2mM, Uricase (Microbial) >150 U/L, Peroxidase (horseradish) >2,500 U/L, Buffer, pH 8.1 ± 0.1, Non-reactive stabilizers.

Reagent Preparation
The reagent is ready to use.

Reagent Storage and Stability
The reagent set is stored at 2-8°C. Under proper storage the reagent will remain stable until the indicated expiration date.

Precautions
1. This reagent set is for in vitro diagnostic use only.
2. The reagent should not be used if: The reagent is turbid or contains obvious microbial growth. The reagent blank has an absorbance of 0.500 or greater at 520nm. A pink color is normal for this reagent.
3. All specimens and controls should be handled as potentially infectious, using safe laboratory procedures. (NCCLS M29-T2)[8]

Specimen Collection and Storage
1. Unhemolyzed serum is recommended.
2. Uric Acid in serum is stable for three days at 2-8°C and up to six months when frozen.[9]
3. Collect specimens per NCCLS document H4-A3.[10]

Interferences
1. Elevated ascorbic acid levels can result in falsely depressed Uric Acid values.
2. Lipemic samples may cause falsely elevated Uric Acid levels.
3. Hemoglobin to 100 mg/dl has been demonstrated to have a negligible effect (<5%) on Uric Acid values. Hemoglobin greater than 100 mg/dl may cause falsely elevated Uric Acid values.
4. Bilirubin to 30 mg/dl has been demonstrated to have a negligible effect (<5%) on Uric Acid results using this method.
5. See Young, et al[11] for other interfering substances.

Materials Provided
Uric Acid Reagent.

Materials Required but not Provided
1. Accurate pipetting devices.
2. Timer.
3. Test tubes/rack
4. Spectrophotometer with ability to read at 520 nm.
5. Heating Block
6. NIST-traceable Uric Acid standard or calibrator
7. Serum controls with known normal and abnormal uric acid values.

Procedure (Automated-General)

Wavelength:	520nm
Assay Type:	Endpoint
Sample/Reagent Ratio:	1:41
Reaction Direction:	Increasing
Temperature:	37°C
Incubation Time:	600 seconds
Low Normal:	2.5 mg/dl
High Normal:	7.7 mg/dl

Procedure (Manual)
1. Label test tubes: "Blank", "Standard", "Control", "Sample", etc.
2. Pipette 1.0 ml of working reagent into each tube.
3. Pre-warm at 37°C for at least five minutes.
4. Add 0.025 ml (25ul) of sample to respective tubes and mix.
5. Incubate all tubes at 37°C for ten minutes.
6. Zero spectrophotometer with reagent blank at 520nm. Read and record absorbances of all test tubes.
7. To determine results see "Calculations".

Limitations
1. If the spectrophotometer being used requires a final volume greater than 1.0ml for accurate reading, use 0.075ml (75ul) of sample to 3.0ml of reagent. Perform the test as described above.
2. The procedure described is linear to 20 mg/dl. Samples with values exceeding 20 mg/dl should be diluted 1:1 with saline, re-assayed, and the results multiplied by two.

3. Lipemic samples will give falsely elevated results and a serum blank must be run. Serum Blank: Add 0.025ml (25ul) of sample to 1.0ml water. Zero spectrophotometer with water. Read and record absorbance and subtract reading from test absorbance. Calculate as usual.

Calibration

Use an NIST-traceable Uric Acid standard (5.0 mg/dl) or serum calibrator. The procedure should be calibrated according to the instrument manufacturer's calibration instructions. If control results are found to be out of range, the procedure should be re-calibrated.

Calculations

A = Absorbance

$$\frac{A\ (Unk)}{A\ (Std)} \times \text{Conc. of Std (mg/dl)} = \text{Uric Acid (mg/dl)}$$

Example: A (Unk) =0.126, A (Std) = 0.100, Conc. of Std = 5 mg/dl.

Then: $\frac{0.126}{0.100} \times 5 = 6.3$ mg/dl

SI Units (mM/L)

To convert to mM/L, multiply the result (mg/dl) by 10 to convert dl to L and divide by 168 (the molecular weight of Uric Acid).

Mg/dl x $\frac{10}{168}$ = mM/L mg/dl x .0595 = mM/L

Example: 6.3mg/dl x .0595 = 0.374mM/L

Quality Control

Serum controls with known normal and abnormal uric acid values should be run routinely to monitor the validity of the reaction. These controls should be run at least with every working shift in which uric acid determinations are performed. It is strongly recommended that each laboratory establish their own frequency of control determination.

Expected Values

2.5 - 7.7mg/dl[9]

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

Performance

1. Assay Range: 0 - 20 mg/dl
2. Comparison: Results obtained with this reagent (y), in 132 samples ranging in Uric Acid from 1.9-10.5 mg/dl, were compared with those obtained in the same samples using a dry-powder reagent (x) based on the same methodology. The correlation coefficient was 0.999 and the regression equation was y=1.00x-0.02. (Sy.x=19.66).
3. Precision: Precision studies were performed following the guidelines contained in NCCLS document EP5-T2.[12]

Within Day (n=20)			Day to Day (n=20)		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
6.0	0.06	1.0	6.1	0.16	2.63
6.9	0.04	0.58	7.2	0.16	2.24
9.9	0.06	0.60	10.2	0.31	3.05

4. Sensitivity: The sensitivity of this reagent was investigated by reading the change in absorbance at 520nm for a saline sample, and two serum samples with known concentrations. Ten replicates of each sample were performed. The results of this investigation indicated that, on the analyzer used, the Uric Acid (Liquid) reagent showed little or no reagent drift on a zero sample. Also, that an absorbance change of 0.015 was approximately equivalent to 1 mg/dl of Uric Acid.

References

1. Folin, D., Dennis, W., J. Biol. Chem. 13:469 (1913).
2. Caraway, W.T., Clin. Chem. 4:239 (1963).
3. Morin, L.G., J. Clin. Path. 60:691 (1973).
4. Morin, L.G., Clin. Chem. 20:51 (1974).
5. Brochner-Mortenson, K., Medicine 19:161 (1940).
6. Klackar, H.M., J. Biol. Chem. 167:429 (1947).
7. Praetorius, E., Poulson, H., Scand. J. Clin. Invest 5:273 (1953).
8. NCCLS document "Protection of Laboratory Workers form Infectious Disease Transmitted by Blood, Body Fluids, and Tissue", 2nd Ed. (1991).
9. Henry, R.J., Clinical Chemistry: Principles and Technics, 2nd Ed., Hagerstown (MD), Harper & Row, pp. 531 & 541 (1974).
10. NCCLS document "Procedures for the Collection of Diagnostic Blood Specimens by Skin Puncture", 3rd Ed. (1991).
11. Young, D.S., et al. Clin. Chem. 21:1D (1975).
12. NCCLS document "Evaluation of Precision Performance of Clinical Chemistry Devices", 2nd Ed. (1992).

Manufactured by Pointe Scientific, Inc.
5449 Research Drive, Canton, MI 48188

European Authorized Representative:
Obelis s.a.
Boulevard Général Wahis 53
1030 Brussels, BELGIUM
Tel: (32)2.732.59.54 Fax: (32)2.732.60.03 email: mail@obelis.net

Rev. 12/09 P803-U7581-01