Pointe Uric Acid Reagent Set (UV)

HORIBA Instruments Incorporated
5449 Research Drive, Canton, MI 48188
Phone: 734-487-8300; (800) 445-9853

Intended Use
For the quantitative determination of Uric Acid in serum using the Yumizen C230 and Yumizen C240 analyzers. For in vitro diagnostic use only.
Rx 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. Yumizen C230 / Yumizen C240 Analyzer
2. Yumizen C230 / Yumizen C240 Operation manual
3. Pointe Chemistry Calibrator, catalog number C7506-50
4. Pointe Chemistry control, catalog number C7592-100

Test Parameters

Test:	URIC	Chemistry:	Uric Acid
Chemistry No.:	0-20	Print Name:	Uric Acid
Reaction Type:	Endpoint	Reaction Direction:	Positive
Pri. Wave:	510 nm	Sec. Wave:	670 nm
Decimal.:	0.1	Samp. Type:	Serum
Blank Time:		Reaction Time:	35 37
Unit:	mg/dL	Incubation Time:	0

	Sample Vol.	Aspirated	Diluent		Reagent Vol.	Diluent	
Standard;	4	uL	uL	uL	180	uL	uL
Decreased;		uL	uL	uL			
Increased;		uL	uL	uL			

Linearity Range (Standard); 0-20			Linearity Limit:		
Linearity Range (Decreased):			Substrate Depletion:		
Linearity Range (Increased):			Mixed Blank Abs.:	- 40000	40000
R1 Blank Abs.:	- 40000	40000	On-board Stability:	30	Day (s)
Blank Response	- 40000	40000	Reagent Alarm Limit:	5	
Twin Chemistry:					

Prozone Check:		
Q1:	Q2:	Q3:
Q4:	PC:	ABS:

Use Qualitative Result:		
	Range:	Flag:

Slope Offset:			
	Slope	Offset	Unit
	1	0	mg/dL

Pretreatment:			
Pretreat Sample Vol.:	uL	Pretreat Reagent Vol.:	uL

Ref. Range:					
Sample Type:	Gender:	Age Range:	Ref. Range:	Critical Range:	Unit:

Pointe
Uric Acid
Reagent Set (UV)

Calibration Setup Parameters

Chem: UA
Calibration Setting
Math Model: Two-Point Linear
Factor: Replicates: 2
Acceptance Limits
Cal Time: 336 hr.
Slope Diff: SD:
Sensitivity: Repeatability: * User Defined
Deter Coeff:
Auto Calib.
☐ Cal Time

Calibrator	Conc.	Pos	Lot No.
Water	0.0	W	
Chem Cal	*	*	

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 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 (Example)

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 its own frequency of control determination. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

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: A study was performed between the Yumizen 200 series analyzers and a similar analyzer and method, resulting in a correlation coefficient of 0.998 and the regression equation was y=1.015x+0.02.
3. Precision: Precision studies were performed using the Yumizen 200 series analyzers following a modification of the guidelines which are 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.%**
6.63	0.31	4.6	7.11	0.14	2.0
9.38	0.16	1.7	10.13	0.20	2.0

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).

Symbol Key

Use by (YYYY-MM-DD)	LOT Lot and batch code
REF Catalog number	Manufacturer
IVD *In vitro* diagnostic medical device	Temperature limitation
Consult instructions for use	**Rx Only:** Prescription Use Only
CE mark	EC REP Authorized representative in the European Community

REF 12-U7581-120

Manufactured by
HORIBA Instruments Incorporated - Pointe Brand
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IVD

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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

Certified to Perform Reagents

The Pointe reagents are certified to be manufactured according to specified parameters. Any Pointe reagent product not meeting specifications through its listed expiration date will be remedied immediately without charge.