Pointe Uric Acid Reagent Set (UV)

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Intended Use
Pointe Uric Acid Reagent Set is intended for research use only quantitative determination of Uric Acid in urine using the Yumizen C230 and Yumizen C240 analyzers.

Clinical Significance
The determination of uric acid in urine is useful in determining the cause of hyperuricemia and accessing risk for formation of kidney stones.[1]

Test Summary
Uric Acid has been determined by phosphotungstate methods,[2] variations of the phosphotungstate method[3] and iron reduction methods.[4,5] The above methodologies are influenced by many substances in their procedures as well as many contaminating substances on glassware, etc.[6] The enzyme Uricase has been widely used for Uric Acid determinations because of its improved specificity.[7,8] 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. 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.
2. All specimens and controls should be handled as potentially infectious, using safe laboratory procedures. (NCCLS M29-T2)[9]

Specimen Collection and Storage
1. Uric Acid in urine can be stored for up to 4 days at 20-25°C if pH > 8.0.[10]
2. Collect specimens per NCCLS document GP16-A3.[11]

Interferences
An extensive list of drugs or other agents interfering with uric acid in urine has been reported by Young, et al[12].

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 Human Urine control set, catalog number P7582-CTL

Test Parameters

Chem:	UA	Chemistry:	Uric Acid
Chemistry No.:	231	Print Name:	UA
Reaction Type:	Endpoint	Reaction Direction:	Positive
Pri. Wave:	510 nm	Sec. Wave:	670 nm
Decimal.:	0.1	Samp. Type:	Urine
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); 0.1-100.0			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:	10
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:

Calibration Setup Parameters

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

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

* User Defined

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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 100 mg/dl.

Calibration
Use an NIST-traceable 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
Urine 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
250-750mg/24 hours[1]
It is strongly recommended that each laboratory establish its own normal range.

Performance
1. Assay Range: 0.1 – 100.0 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 0.9842 and the regression equation was y=1.076x-1.45.
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.[13]

Within Day (n=20)

Mean	S.D.	C.V.%
7.8	0.31	4.0
19.5	0.37	1.9
44.5	0.44	1.0

4. Sensitivity: Limit of blank (LOB): 0.06mg/dl

References

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9. NCCLS document "Protection of Laboratory Workers form Infectious Disease Transmitted by Blood, Body Fluids, and Tissue", 2nd Ed. (1991).
10. Use of anticoagulants in diagnostic laboratory investigations. WHO publication WHO/DIL/LAB/99.1Rev.2, p.49 (2002).
11. NCCLS. Urinalysis and collection, transportation and preservation of urine specimen; Approved guideline -3nd Edition, NCCLS document GP16-A3 (2009)
12. Young DS. Effects of Drugs on Clinical Laboratory Tests. 4th Edition, Washington, DC, AACC Press (1995) 3: 621-622.
13. 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
Temperature limitation	Consult instructions for use

Research use only

REF 12-U7581-120

Manufactured by
HORIBA Instruments Incorporated - Pointe Brand
5449 Research Drive Canton, MI 48188

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.

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