POINTE SCIENTIFIC
A MEDTEST COMPANY

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 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 procedures uses the coupling of 4-aminoantipyrine (4-AAP), 2-hydroxy-3,5-dichlorobenzenesulfonate (HDCBS), and hydrogen peroxide in the presence of peroxidase to yield a chromagen measured spectrophotometrically.

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 chromogen that is measured at 520 nm. The absorbance is proportional to the concentration of uric acid in the sample.

Reagent Composition
When combined the reagent contains: 4-AAP >0.2mM, HDCBS 2mM, Uricase (microbial) >150 U/L, Peroxidase (horseradish) >2,500 U/L, Buffer, pH 7.5 ±0.1, Non-reactive stabilizers.

Reagent Preparation
The reagents are ready to use.

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

Precautions
1. This reagent is for *in vitro* diagnostic use only.
2. The reagent should not be used if the reagent is turbid or contains obvious microbial growth.
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. Bilirubin to 7 mg/dl has been demonstrated to have a negligible effect (<10%) on uric acid results using this method.
4. See Young, et al[11] for other interfering substances.

Materials Provided
Uric Acid reagent.

Materials Required but not Provided
1. Controls.
2. Calibrator.
3. Beckman Coulter AU™ analyzer.
4. Application and instrument manuals.

Procedure (Beckman Coulter AU™400 application)

SPECIFIC TEST PARAMETERS

TEST NUMBER: # TEST NAME: UA ∇ TYPE: Serum ∇ OPERATIONAL: Yes ∇
SAMPLE VOL.: 4 DIL. VOL.: 0 PRE-DILUTION RATE: 1
REAGENTS: R1 VOLUME: 150 DIL. VOL.: 0 MIN. OD MAX. OD
R2 VOLUME: 30 DIL. VOL.: 0 L H
REAGENT OD LIMIT:
WAVELENGTH: PRI. 540 ∇ SEC. 700 ∇ FIRST L: 0.000 FIRST H: 0.600
METHOD: END ∇ LAST L: 0.000 LAST H: 0.600
REACTION SLOPE: + ∇ DYNAMIC RANGE:
MEASURING POINT 1: FIRST: 9 LAST: 24 L: # H: #
MEASURING POINT 2: FIRST: LAST: CORRELATION FACTOR:
LINEARITY: % A: 1.000 B: 0.000
NO LAG TIME: ∇ ON BOARD STABILITY PERIOD: #

SPECIFIC TEST PARAMETERS

VALUE FLAG: # ∇ LEVEL L: # LEVEL H: #

NORMAL RANGES:		AGE L		AGE H			
	SEX	YEAR	MONTH	YEAR	MONTH	L	H
○ 1.	# ∇	#	#	#	#	#	#
○ 2.	# ∇	#	#	#	#	#	#
○ 3.	# ∇	#	#	#	#	#	#
○ 4.	# ∇	#	#	#	#	#	#
○ 5.	# ∇	#	#	#	#	#	#
○ 6.	# ∇	#	#	#	#	#	#
7.	NONE SELECTED					#	#
8.	OUT OF RANGE	L	H			#	#

PANIC VALUE: # # UNIT: mg/dl DECIMAL PLACES: 1

CALIBRATION SPECIFIC PARAMETERS

CAL TYPE: AB ∇ FORMULA: Y=AX+B ∇ COUNTS: 2 PROCESS: CONC. ∇

	CAL. NO.	OD	CONC.	FAC/OD-L	FAC/OD-H
POINT 1.	#		#	-9999999	9999999
POINT 2.					
POINT 3.					
POINT 4.					

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POINT 5.		
POINT 6.		
POINT 7.		
1-POINT CAL. POINT:	○	WITH CONC-0
MB TYPE FACTOR:		CALIBRATION STABILITY PERIOD: #

#: User-Defined

The above reagent parameters are intended to serve as a guide for use with Pointe Scientific, Inc. reagent. The parameters are based on data generated by Pointe Scientific, Inc. Please note: These parameters should be used in conjunction with your laboratory Quality Control Program for validation.

NOTE: For other instrument specific applications please contact Pointe Scientific, Inc. Technical Service Department at 1-800-445-9853

Limitations

1. 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.
2. Lipemic samples will give falsely elevated results and a serum blank must be run.

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).

$$\text{mg/dl} \times \frac{10}{168} = \text{mM/L} \qquad \text{mg/dl} \times .0595 = \text{mM/L}$$

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

Calibration

Follow instrument application instructions for calibration. Refer to instrument manual instructions for calibration procedures and frequency. It is recommended that each laboratory determine its own frequency of calibration.

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

2.5-7.7 mg/dl.[9]

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

Reagent Performance

1. Linearity: 0-20 mg/dl.
2. Comparison: A comparison study performed between the Beckman Coulter AU™400 and Roche Hitachi 717 using this method resulted in a correlation coefficient of r = 0.993 and a regression equation of y=0.974x + 1.07. (n = 37, range 2.9 – 12.5 mg/dl)
3. Precision:
   Within - day precision study was performed using three levels of material.
   Between - day precision study was performed using two levels of control material assayed over a 20 day period with 2 runs per day and 2 replicates per run.

Within Day (N=20)			Day to Day		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
2.2	0.02	0.91	4.8	0.14	2.80
7.9	0.10	1.30	9.1	0.20	2.20
12.8	0.12	0.94			

   Precision and Linearity studies were performed following modifications of CLSI Protocols EP-5 and EP6[12] using a Beckman Coulter AU™400 analyzer.
4. Sensitivity:
   The sensitivity of this reagent was investigated by reading the change in absorbance at 540/700 nm 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 reagent showed little or no reagent drift on a zero sample. Also, that an absorbance change of 0.015 was approximately equivalent to mg/dl of uric acid.

References

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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. CLSI protocols EP5-A2, Vol. 24 No. 25, 2nd Ed, (2004) and CLSI EP6-A, Vol. 23 No. 16 (2003)

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