Uric Acid Reagent Set

Intended Use
For the quantitative determination of Uric Acid in serum using the Mindray BS-480 analyzer. 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. Manufacturer studies have shown reagent is stable for 30 days once placed in the refrigerated reagent carousel (2-10°C), however reagent stability may vary based on individual laboratory conditions.

Precautions and Hazards
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]

Hazards:
Hazard Classifications: Specific target organ toxicity, single exposure; Respiratory tract irritation (Category 3)
Hazard Statements: H335: May cause respiratory irritation
Precautionary Statements: **Prevention:** P261: Avoid breathing dust/fume/gas/mist/vapors/spray. P271: Use only in a well-ventilated area **Response:** P304+P340: IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. P312: Call a POISON CENTER or doctor/physician if you feel unwell. **Storage:** P403 + P233: Store in a well-ventilated place. Keep container tightly closed. **Disposal:** P501: Dispose of contents into sewer system after diluting with large volumes of water, if in accordance with local regulations. **Refer to the Safety Data Sheet for this product (SDS-UA600) available at www.medtestdx.com.**

Signal Word: Warning

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 78.7 mg/dl has been demonstrated to have a negligible effect (<10%) 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 (<10%) 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. Mindray BS-480 Analyzer
2. BS-480 Operation manual
3. Chemistry Calibrator, catalog number CHEC480
4. Chemistry control, catalog number CHEQ480

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.

Calibration

Use MedTest DX Chemistry Calibrator (Catalog Number CHEC480). The procedure should be calibrated according to the instrument manufacturer's calibration instructions. If control results are found to be out of range, the test may need to be re-calibrated. Under typical operating conditions manufacturer calibration stability studies have shown the calibration curve will be stable for at least 14 days.

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: 0.0 – 20.0 mg/dl
2. Correlation: A study was performed between the Mindray BS-480 and a similar analyzer using this method, resulting in the following:

Method	Uric Acid
N	100
Mean Uric Acid (mg/dL)	8.20
Range (mg/dL)	0.2-19.8
Standard Deviation	4.58
Regression Analysis	y = 0.988x – 0.01
Correlation Coefficient	0.9977

3. Precision: Precision studies were performed following a modification of the guidelines contained in the NCCLS document EP5-T2.[12]

Within Day

Sample	LOW	MID	HIGH
N	20	20	20
Mean	4.89	9.06	16.34
Standard Deviation	0.04	0.05	0.05
Coefficient of Variation (%)	0.7%	0.5%	0.3%

Day to Day

Sample	LOW	MID	HIGH
N	40	40	40
Mean	4.77	9.00	16.44
Standard Deviation	0.16	0.25	0.46
Coefficient of Variation (%)	3.4%	2.8%	2.8%

4. Sensitivity: 2SD limit of detection (95% Conf) = 0.0 mg/dL

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).

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Uric Acid Reagent Set

CHEMISTRY PARAMETERS

Chem:	URIC	No.:	231	Sample Type:	Serum
Chemistry:	Uric Acid			Print Name:	URIC
Reaction Type:	End Point			Reaction Direction:	Positive
Pri Wave:	505			Sec Wave:	660
Unit:	mg/dL			Decimal	0.1
Blank Time:	10 12			Reaction Time:	47 49

	Sample Vol.	Aspirated	Diluent	Reagent Vol.	Diluent
Standard:	2.7 ul	--- ul	--- ul	R1: 120 ul	--- ul
Decreased:	--- ul	--- ul	--- ul	R2: --- ul	-- ul
Increased:	--- ul	--- ul	--- ul	R3: --- ul	-- ul
	☐ Sample Blank	☑ Auto Rerun		R4: --- ul	--- ul

Slope/Offset Adjustment

Slope: 1 Offset: 0

Linearity Range (Standard)	0 20	Linearity Limit:	
Linearity Range (Decreased)	____ ____	Substrate Depletion:	
Linearity Range (Increased)	____ ____	Mixed Blank Abs:	
R1 Blank Abs:	____ ____	Uncapping Time	
Blank Response:	____ ____	Reagent Alarm Limit:	
Twin Chemistry:		☐ Enzyme Linear Extension	

☐ Prozone Check ○ Rate Check ○ Antigen Addition

Q1: Q2: Q3: Q4:

PC: ABS:

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CALIBRATION PARAMETERS

Calibrator Definition

Calibrator:	*	Lot No.:	*
Exp Date:	*		

Carousel	Pos
Sample Carousel 1	*
Sample Carousel 2	
Sample Carousel 3	

Reagent/Calibration

Calibrator	Pos	Lot No	Exp Date	Chem	Conc	Unit
Water	W	*	*	URIC	0	mg/dL
Chemistry Calibrator	*	*	*	URIC	*	mg/dL

Calibration Setup

Chem: URIC

Calibration Settings

Math Model:	Two-Point Linear		
Factor:		Replicates:	2

Acceptance Limits

Cal Time:	*	Hour	
Slope Diff:	---	SD:	---
Sensitivity :	---	Repeatability:	---
Deter Coeff:	---		

Auto Calib.

☐ Bottle Changed ☐ Lot Changed ☐ Cal Time

It is recommended that two levels of control material be assayed daily.
* Indicates user defined parameter.

REF UA480

Manufactured for MedTest DX
5449 Research Drive Canton, MI 48188

2°C 8°C

IVD

Symbol Key

Use by (YYYY-MM-DD)	LOT Lot and batch code	REF Catalog number	Manufacturer
Temperature limitation	Consult instructions for use	IVD *In vitro* diagnostic medical device	