

Technical method

A new automated uric acid assay

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Currently, the most popular automated method for the determination of serum uric acid is the automated method modified by Musser and Ortigoza (1966) for the Technicon® AutoAnalyzer system. While this method is considered reliable, it suffers from the following deficiencies: (a) it shows non-linearity with dilutions of aqueous uric acid standards and is thus dependent on costly reference sera as secondary standards; (b) it has been found to give results which are 14% higher than the manual uricase reference method of Liddle *et al.* (1959) according to Technicon's literature², and approximately 0.6 mg/dl (0.036 mmol/l) higher according to Itiaba *et al.* (1975); (c) recovery is incomplete and aqueous standards containing formaldehyde, which is traditionally used as a preservative, give grossly incomplete recovery (Klein and Sheehan, 1937); and (d) a high concentration of costly sodium tungstate is used as one of the reagents in this method.

In a preliminary study with the SMA 12/60 (unpublished data), we observed non-linearity of aqueous uric acid standards introducing errors up to 30%. On calibrating the SMA 12/60 with the aqueous uric acid standards, similar non-linearity persisted affecting the sera values.

In order to overcome some of these difficulties generally encountered with the continuous-flow automated methods, we selected the unique reagent system of Jung and Parekh (1970) for purposes of automation. As a result of our investigation we have been able to present in this paper an automated uric acid assay, which is free of most of the aforementioned problems. More significantly, the applicability of primary uric acid standards (with or without formaldehyde) in the proposed assay has enhanced the analytical accuracy of uric acid measurements in clinically important biological fluids

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besides sera, while significantly lowering the cost per test.

Material and methods

AutoAnalyzer modules from Technicon Corporation, as indicated in Fig. 1, were used for this study. With the exception of urea, phosphotungstic acid reagent, and the detergent FC 134, all chemicals are of reagent grade.

FC 134

This is available from Environmental Specialties, Inc, Schenectady, New York, USA.

2% TRISODIUM PHOSPHATE (TSP)

Dissolve 10 g (0.0263 moles) of sodium phosphate tribasic ($\text{Na}_3\text{PO}_4 \cdot 12 \text{H}_2\text{O}$) and 0.5 ml of FC 134 in 500 ml of water.

CARBONATE-UREA-TRIETHANOLAMINE (CUTE)

Dissolve 100 g (0.943 moles) of anhydrous sodium carbonate, 200 g (3.33 moles) of urea, 50 g (0.335 moles) of triethanolamine, and 1 ml of FC 134 in water to 1 litre.

CUTE/2—TSP

Mix equal volumes of CUTE and 2% TSP.

PHOSPHOTUNGSTIC ACID REAGENT

This is commercially available from Environmental Specialties, Inc, Schenectady, New York, USA.

URIC ACID STOCK STANDARD 100 mg/dl

(5.9 mmol/l) WITH FORMALDEHYDE

Dissolve 100 mg (0.0014 moles) of lithium carbonate in 50 ml of water at approximately 60°C in a beaker and add 100 mg (0.0006 moles) of uric acid (NBS No. 914). Stir until dissolved, warming further if necessary. Transfer the contents with washings to a 100 ml volumetric flask. Add 4 ml of formaldehyde (37-40%) and then slowly add 1 ml of glacial acetic acid. Bring to volume and store in the refrigerator.

URIC ACID STOCK STANDARD 100 mg/dl

WITHOUT FORMALDEHYDE

Prepare as above, but without formaldehyde or acetic acid.

WORKING STANDARDS

Dilute Stock Standard 1:10 (v/v) with water to

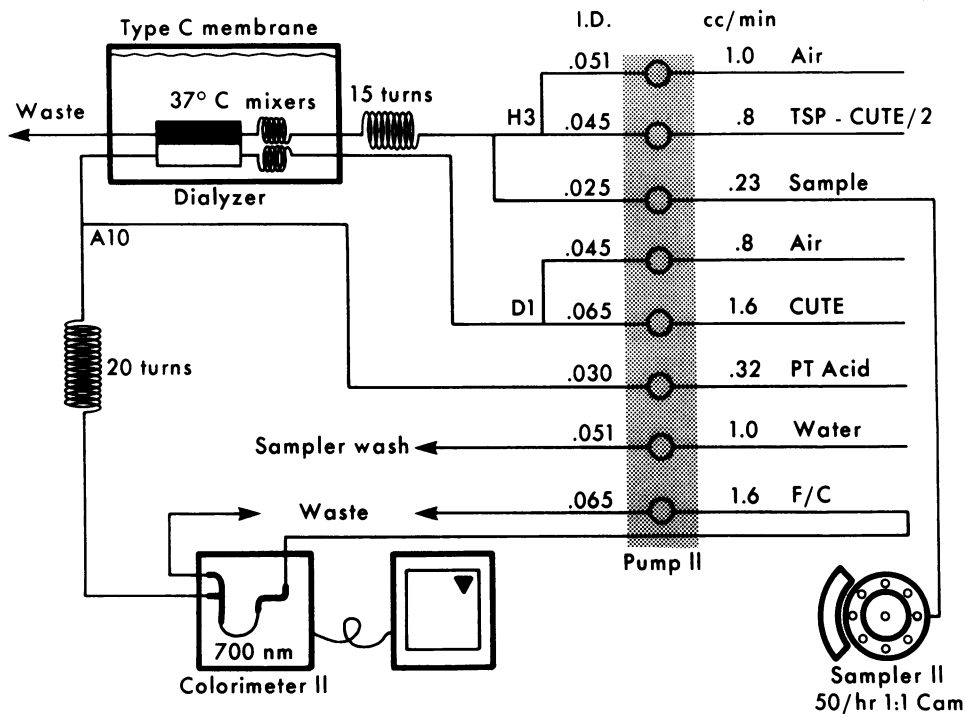


Fig. 1 Flow diagram for the proposed automated uric acid assay.

prepare 10 mg/dl (0.59 mmol/l) standard and then dilute aliquots of 10 mg/dl standard further to prepare 2.5, 5.0, and 7.5 mg/dl (0.148, 0.295, and 0.443 mmol/l) working standards.

Results

Several manifolds and reagent combination were studied before the final system was arrived at. Aqueous standards were used in all studies. Initially, serum and TSP were mixed and dialysed against CUTE with subsequent addition of phosphotungstic acid reagent to the recipient stream. This produced considerably raised serum uric acid values. We found that mixing of serum and 50% dilute CUTE in water, with or without TSP, before dialysis against CUTE produced values which were comparable with Technicon SMA 12/60 automated uric acid values. However, results from the manual method of Jung and Parekh (1970) are lower. We then observed that lower values are obtained if the volume on the recipient side of the dialyser is increased with respect to the volume on the sample side. This led to the current manifold (Fig. 1) which gives good agreement with the parent method.

Correlation with the manual method, as shown in Fig. 2, yields a slope of 1.03, Y intercept of 0.04

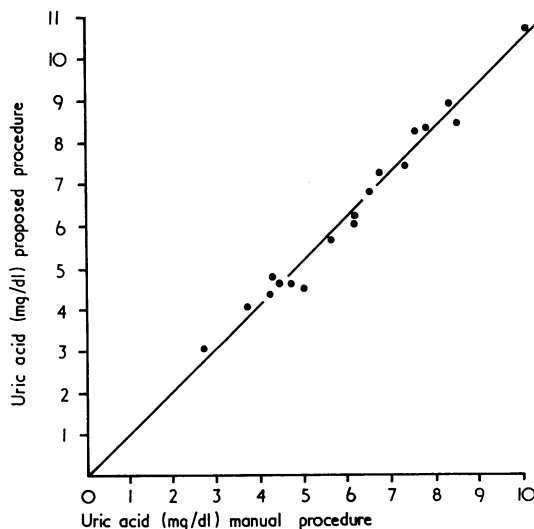


Fig. 2 Scattergram comparing the proposed automated assay with the manual Jung-Parekh uric acid method. Conversion: traditional units to SI—uric acid 1 mg/dl ≈ 0.059 mmol/l.

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Technical method

mg/dl (0.0024 mmol/l), and a correlation coefficient of 0.991. A correlation of the proposed method to the SMA 12/60 procedures gives a correlation coefficient of 0.992 and a slope of 1.01 but a Y intercept of -0.24, indicating that the SMA procedure gives approximately 0.25 mg/dl (0.0148 mmol/l) higher values than the proposed method (Fig. 3). A standard curve is illustrated in Figure 4. The absorbance values are directly proportional to concentration through 15 mg/dl (0.885 mmol/l) uric acid. Sample interaction in this system is approximately 2%, as shown by the fact that a 2.5 mg/dl (0.148 mmol/l) standard following a 10 mg/dl

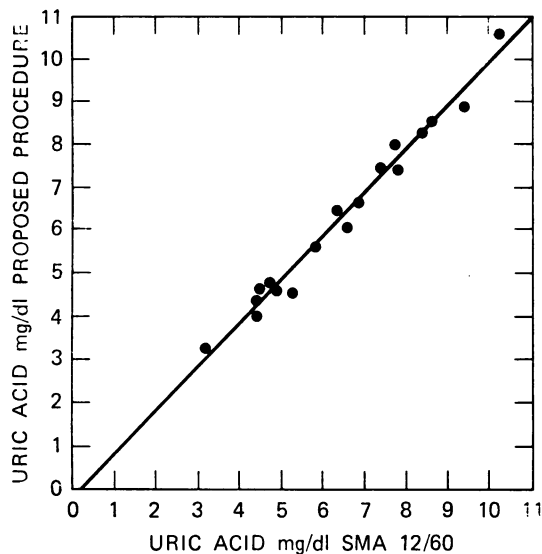


Fig. 3 Scattergram comparing the proposed automated assay with the SMA 12/60 procedure.

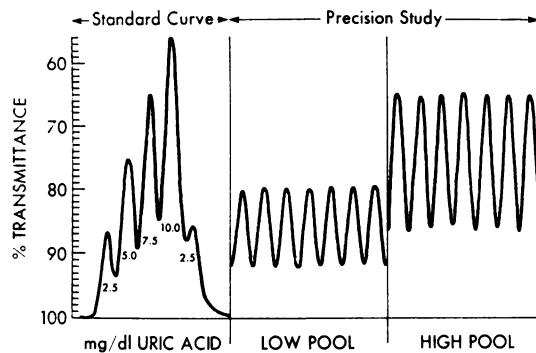


Fig. 4 Standard curve, sample interaction, and precision for proposed automated uric acid assay.

(0.59 mmol/l) standard appears to be 2.7 mg/dl or 0.159 mmol/l (Fig. 4). The precision of the method is also illustrated in Figure 4. The coefficient of variations were 1.56% and 0.75% for 4.1 mg/dl (0.242 mmol/l) and 7.3 mg/dl (0.431 mmol/l) serum uric acid concentrations, respectively.

In order quantitatively to investigate the effect of the presence of formaldehyde in the proposed automated assay, the aqueous standards with or without formaldehyde were used in these studies (Table). The preparation with formaldehyde did not affect the absorbance (peak height) of the standards, whereas 2-month-old non-formaldehyde standards evidenced some deterioration even when stored at 4°C. However, on addition of uric acid containing formaldehyde to serum, only 40% is recovered even when the total uric acid concentration is compared to the lowered value of the serum mixed with identical concentrations of formaldehyde without added uric acid. When the total uric acid value is compared

Table Recovery of uric acid

Serum pool (0.9 ml) mixed with 0.1 ml of	Added uric acid (mg/dl)§	Non-formaldehyde		Formaldehyde			
		Uric acid (mg/dl)	% Recovery compared to		Uric acid (mg/dl)	% Recovery compared to	
			Water control	Blank control‡		Water control	Blank control‡
Water	0.0	3.80		3.66			
Water	0.0	3.98		3.80			
Blank (for 25 mg/dl std)†	0.0	4.01	(103)	1.36	(37)		
25 mg/dl uric acid std*	2.5	6.42	101	96	2.35	-55	40
Blank (for 50 mg/dl std)†	0.0	4.00	(103)	1.15	(31)		
50 mg/dl uric acid std*	5.0	9.03	103	100	3.15	-12	40

*Preparation of standards: 0.9 ml water is added to 0.1 ml of 25, 50, 75, and 100 mg/dl standards.

†Blanks are prepared like their corresponding standard from a stock blank containing no uric acid but only lithium carbonate or lithium carbonate, formaldehyde, and acetic acid.

‡Recovery compared to blank control indicates recovery of uric acid standard after the formaldehyde effect on endogenous serum uric acid has been accounted for.

§Conversion: traditional units to SI—uric acid 1 mg/dl ≈ 0.059 mmol/l.

Parentheses indicate recovery of endogenous uric acid.

with a water blank, a negative recovery of (–) 55% is obtained (Table). When formaldehyde is added to serum in the concentrations that would be obtained with the addition of 2.5 mg/dl (0.148 mmol/l) uric acid standard containing formaldehyde, the serum value is lowered to 37% of its value, which is similar to 40% recovery of uric acid added to serum compared with the blank control (Table). Recoveries are about 100% on employing uric acid standards without formaldehyde in this assay.

L-ascorbic acid, which is known to interfere with uric acid methods, is destroyed by the initial mixing of serum with TSP and CUTE for three minutes before dialysis. The interference of 10 mg/dl (0.567 mmol/l) ascorbic acid is equivalent to 0.1 mg/dl (0.0059 mmol/l) uric acid, which is similar to that observed by use with the Technicon SMA 12/60 procedure. Interferences by sodium salicylate (40 mg/dl or 2.22 mmol/l), *L*-tryptophan (10 mg/dl or 0.49 mmol/l), and glucose (400 mg/dl or 0.022 mol/l) were negligible in both the proposed and the SMA 12/60 procedures.

The cost of uric acid reagents and secondary serum standards for eight hours SMA 12/60 operation per day for 365 days would be approximately \$3200. Considering the generous discount usually available to bulk consumers, the cost per year would still be about \$2200. Of this amount, about half the cost is for sodium tungstate reagent and about one-quarter the cost is for serum standards. The comparable cost for the proposed automated procedure as described would be approximately \$1050 per annum, that is, one-third to one-half the cost of the SMA 12/60 operation.

Discussion

The proposed method correlates well with its parent, the method of Jung and Parekh (1970), which has been shown to produce values between those of Brown (1945) and Liddle *et al.* (1959), and has been recommended as a method of choice by Sunderman¹ since its publication in 1970. However, while its correlation is $r = 0.99$, the values obtained are 3% higher than the manual method. It still yields uric acid values which are 0.24 mg/dl (0.0142 mmol/l) lower than those from the SMA 12/60 automated procedure, which has been reported to give approximately 0.6 mg/dl (0.0354 mmol/l) higher values than those from the uricase reference method (Liddle *et al.*, 1959). Based on the observation that a higher ratio of recipient stream to sample stream volume lowers uric acid values, one might expect that the

difference between the proposed automated method and its parent could be further decreased by increasing the volume of the recipient stream. But this would be accomplished only at the expense of a significant loss of sensitivity.

Like Klein and Lucas (1973), but unlike Klein and Sheehan (1973), we found no difference in absorbance values for uric acid standards with or without formaldehyde. This indicates that formaldehyde itself does not directly influence uric acid assay or its dialysis rate. Our data (Table) are in agreement with those of Klein and Lucas (1973) and Klein and Sheehan (1973) that the presence of formaldehyde leads to incomplete recovery of endogenous as well as exogenously added uric acid in serum. Our findings do not agree with those of Brown and Frier (1967), who found that formaldehyde also interfered by increasing the uric acid values up to about 10%. Reports in the literature have implicated the effect of formaldehyde on proteins as being responsible for the above problems. Our results indicate that the addition of formaldehyde lowers the endogenous and the added uric acid about equally, for example, 37% and 40% respectively (Table). Such quantitative agreement supports the conclusion that the interaction of formaldehyde with proteins is the common mediator of this effect on the analyses of endogenous serum uric acid, and uric acid added to serum.

Although aqueous standards without formaldehyde gave 100% recovery when added to serum, we recommend the use of formaldehyde-containing standards because of their stability. Since the absorbance values of standards with or without formaldehyde in the proposed method are equal, the accuracy of the proposed method (as determined by recovery) should be considered good, because the sample values obtained will be identical with those obtained using non-formaldehyde standards. It is only necessary to prevent mixing of sera and formaldehyde, and, to that end, inserting a water blank between standards and serum samples is adequate.

Reliable results are obtained with the proposed procedure employing primary aqueous uric acid standards, while the SMA 12/60 procedure is dependent on the so-called reference sera which may eliminate or mask difficulties involving constituents such as proteins. One such difficulty involves inaccurate analyses of non-protein or low-protein biological fluids, such as urine or synovial fluid. In this regard, the improved standardisation achieved in the proposed method is of considerable analytical significance.

In conclusion, the proposed automated method is shown to be precise and accurate. It agrees more closely with the reference method than does the

¹Monthly Report, Proficiency Test Service, Institute for Clinical Science, Philadelphia Pa, June 1976, pp. 6-7.

Technical method

SMA 12/60 method. Its improved standardisation is of considerable clinical importance. Its economy (cost per test is less than half of that with SMA 12/60) and other advantages merit consideration for its general application to the determination of uric acid in serum and other biological fluids. Furthermore, the principles outlined here can also be adapted to any continuous-flow automated system.

Preliminary experiments on this procedure were done with the assistance of Mr R. C. King at the Department of Pathology, Upstate Medical Center, Syracuse, NY, USA. We thank Dr von Redlich for a helpful review and suggestions and appreciate the excellent editing and preparation of this manuscript by Mrs Jean P. Nelson and Miss Janice Perry.

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Letters to the Editor

A sensitive urine-test for monitoring the ingestion of isoniazid

Ellard and Greenfield (1977) described in your Journal a highly sensitive 'new isonicotinic acid method' for controlling isoniazid ingestion in urine. This new method is one of several modifications of the chemical procedure published 19 years ago by Nielsch (1958). His basic principle has also been utilised by others in the design of tests for the detection of isonicotinic acid in urine (Kasik *et al.*, 1962; Belles and Littleman, 1962; Tuberculosis Prevention Trial, 1967). All these procedures are suitable for the control of the daily intake of INH provided 300-400 mg therapeutic doses are administered. Our control test (Eidus and Hamilton, 1964a, b), to which reference is made by Ellard and Greenfield (1977), detects *acetylisoniazid*, the main metabolite of INH, in urine. The *acetylisoniazid* method is considered as sensitive as the tests introduced by Kasik *et al.* (1962) and Belles and Littleman (1962). The advantage of the Eidus-Hamilton (1964a) procedure is its great simplicity. It may be carried out by laboratory workers, without technologist qualification, trained at the job. Employing automatic pipettes, approximately 250 tests can be completed

in one hour. The sensitivity of the *acetylisoniazid* procedure could be increased; however, such modification would affect the speed and ease of the performance. Because of its simplicity, the *acetylisoniazid* method has in the last decade been extensively used by the British Medical Research Council in their tuberculosis chemotherapeutic trials undertaken in Europe, South-East Asia, and Africa.

The test detects 10-15 $\mu\text{g/ml}$ *acetylisoniazid* in urine (Eidus and Hamilton, 1964a; Venkataraman *et al.*, 1965). Hence it was most surprising to note that in the experiments of Ellard and Greenfield (1977) urine samples containing 30 and 40 $\mu\text{g/ml}$ *acetylisoniazid* produced with our method positive readings in only 82% and 91%, respectively, of the specimens.

A volunteer study was also conducted by Ellard and Greenfield in which oral doses of 50 and 100 mg isoniazid were taken by the participants and urine specimens were collected at various intervals for the performance of the *acetylisoniazid* procedure and 'the new isonicotinic acid test'. In this trial, the new test exhibited, with a dose of 100 mg INH, 96% and 34% positive readings in urine samples collected between 23-24 and 47-48 hours, respectively, after drug administration. If the authors intended to prove that their method is suitable for checking daily INH intake, at least therapeutic doses should

also be employed in the volunteer trial. The experimental design of this study does not allow conclusions to be drawn. It seems from the results obtained with 100 mg isoniazid in volunteers that the new method is not only cumbersome but far too sensitive to control the regularity of daily INH self-medication.

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