Portable Electrochemical Blood Uric Acid Meter

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We describe a new portable uric acid (UA) meter, called the UASure[®] (Apex Biotechnology Corp., Hsinchu, Taiwan). The UASure[®] is an electrochemical blood UA meter designed for fast monitoring of UA concentrations in one drop of capillary blood using an electrochemical test strip. We compared the UASure[®] with the standard method, the Hitachi 7600 modular system (Hitachi, Tokyo, Japan), in 146 volunteers (average age 62.5 ± 12.8 years). Of these, 65 were known hyperuricemic subjects, 17 of whom received medical therapy. The patients donated their capillary and venous blood samples in random order. Capillary blood and one drop of venous blood were tested immediately by the UASure[®]. The venous blood in the test tube was sent to

the central laboratory for serum UA measurement by the Hitachi 7600. The intra-assay coefficients of variation (CVs) of the UASure[®] were 4.79%, 5.77%, and 3.08% at UA levels of 5.8, 7.1, and 13.5 mg/dl, respectively. The UA concentrations tested by the UASure[®] correlated well with those by the Hitachi 7600 ($r = 0.87$ in venous sampling and $r = 0.78$ in capillary sampling, $P < 0.001$). The intraclass correlation was good for venous samples by the UASure[®] ($r_t = 0.84$, 95% CI 0.82–0.90), somewhat below the meaningful criterion for capillary samples by the UASure[®] ($r_t = 0.77$, 95% CI 0.69–0.83). UASure[®] with venous sampling is interchangeable with the standard method for UA measurement. J. Clin. Lab. Anal. 16:109–114, 2002. © 2002 Wiley-Liss, Inc.

Key words: electrochemistry; electrodes; uric acid

INTRODUCTION

Uric acid (UA) is the end product of purine metabolism in humans (1). In Taiwan the prevalence of gout is 0.16% in rural areas and 0.67% in urban areas (2). The importance of serum UA as a risk factor for cardiovascular disease has been debated for nearly 50 years (3). In recent years one prospective study supported UA as an independent risk factor (4), while another did not (5). UA is associated with hypertension, hyperlipidemia, and glucose intolerance, which play a causal role in cardiovascular disease (3). Therefore, asymptomatic hyperuricemic subjects should be encouraged to make changes in diet and lifestyle (3). The ability to monitor serum UA levels would be helpful in the follow-up of hyperuricemic patients to give them adequate feedback and treatment guidelines.

Methods based on uricase and mass fragmentography have

been proposed as reference methods (6). However, mass fragmentography is not suitable for routine clinical practice, and is only applied to offer a certified reference method for calibration purposes (6). Uricase methods with colorimetry or spectrophotometry are much more popular in clinical practice. The blood is centrifuged to serum (or plasma) and then sent to an automated analyzer, which is widely available in hospital laboratories.

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We evaluated a new, portable UA meter called the UASure® (Apex Biotechnology Corp., Hsinchu, Taiwan), which is based on amperometric electrochemistry using a disposable, non-enzymatic potassium ferricyanide electrode strip with only one drop of whole blood. It is convenient for clinical use because data are shown in 30 sec, which saves time for both patients and doctors. In this study we compared the UASure® with the standard method of serum UA measurement, the Hitachi 7600 modular system (Hitachi, Tokyo, Japan), to determine whether the new machine is interchangeable with the standard method in clinical use.

MATERIALS AND METHODS

Subjects

The study included 146 volunteers (102 males and 44 females) recruited at Taipei Veterans General Hospital from Oct 2000 to Feb 2001. Their average age was 62.5 ± 12.8 years. Of these, there were 65 known hyperuricemic subjects, 17 of whom received medical therapy. There were 76 known diabetic subjects (seven on diet control, and 69 on oral hypoglycemic agents) and 71 known hypertensive patients (58 on antihypertensive agents). Their hematocrits were within the range of 35–50%. The Institutional Review Board of this hospital approved the study, and all of the subjects signed the informed consent form before examination.

Instrument

The UASure® is a small ($10 \times 6 \times 2$ cm), battery-powered, lightweight instrument (Fig. 1) designed for fast, self-monitoring of capillary UA concentration. It is activated by inserting a disposable electrode strip into the lower part of the meter. Measurement is initiated by placing one drop (6 μ l) of capillary blood on a reaction zone on the test strip, and is based on amperometric electrochemistry using a non-enzymatic, printed, two-layer, carbon-pasted, dry-electrode strip. At 300 mV and pH = 7.0–10.0, the electrons are transferred to an underlying electrode through the major electron mediator ($K_3Fe(CN)_6$, potassium ferricyanide). The current detected by the electrode on the test strip is in proportion to the UA concentration (Fig. 2), which is displayed on a large digital screen in mg/dl. The meter automatically stores the results of the last 10 tests. The measuring range of the meter is 2–20 mg/dl. Results above or below this are displayed as “Hi” or “Lo.” The disposable electrode test strips are supplied in individually sealed aluminum foil packs, which are stored at room temperature. The operation temperature is 18–38°C.

Sample Collection and Blood UA Measurement

The linearity, analytic recovery, and interference evaluations were performed at the Apex Biotechnology Corporation. The uricase method (EPAC 6140 (Eppendorf, Hamburg,

Fig. 1. The UASure® blood UA meter with a test strip.

Germany) and UA Plus 1661868 Kit (Roche, Postfach, Switzerland)) was used as the reference method. The precision determination and the clinical study were performed at Taipei Veterans General Hospital. The patients donated their capillary and venous blood samples in random order. A technician performed the capillary blood sampling with lancets, and then immediately measured the UA levels by the UASure®. The venous blood samples were also immediately tested by the UASure® and collected into test tubes for central laboratory UA measurements by the standard method (Hitachi 7600).

Precision Evaluation

Venous whole blood in ethylene diamine tetra-acetic acid (EDTA) was used for the determination of precision. All measurements were performed within 20 min of sampling to minimize assay errors. Twenty replicates of each of the three different venous blood samples were measured by the UASure® for a precision test that was expressed as the intra-assay coefficient of variation (CV). Ten samples were measured by three different UASure® meters with the same batch of reagent strips, and the other 10 were measured by three

Fig. 2. Current-to-time plotting in different UA concentrations. A sample volume of 8 μ l whole blood was pipetted to a reactive area of the dry-electrode strip and incubated for 20 sec. The current-to-time plotting was recorded at +300 mV vs. the Ag/AgCl reference electrode by BAS CV-50W (West Lafayette, IN) voltammetric analyzer (function mode: time base). The UA concentrations in whole blood were (a) 5.96, (b) 8.58, (c) 11.16, (d) 14.06, and (e) 16.98 mg/dl.

different batches with the same UASure[®] meter to evaluate the intermeter and interreagent strip batch variability (7).

Linearity

A series of dilutions were made by mixing two venous samples: one with a high (21.9 mg/dl) and the other a low (2.3 mg/dl) UA concentration (reference method). We evaluated the relationship between the detected current values from the electrode and UA concentrations of a series of diluted venous samples. The current values of the electrode were detected by a BAS CV-50W (West Lafayette, IN) voltammetric analyzer. In addition, we directly determined the relationship between expected values (reference method) and the actual values by UASure[®] in a series of diluted venous samples (3.5–18.5 mg/dl).

Analytical Recovery

Analytical recovery was assessed by adding UA (U-0881; Sigma Chemical Co., St. Louis, MO), 5 mg/dl or 10 mg/dl, to venous samples from healthy volunteers with normal hematocrits. A total of 10 replicates were assayed.

Interference

We assessed the putative interfering drugs and endogenous metabolites in our interference study. The drugs tested included ketoprofen, diclofenac, indomethacin, amiloride, colchicine, atenolol, allopurinol, glibenclamide, metformin, tolbutamide, and methyl-dopa (all from Sigma). They were all dissolved in dimethyl sulfoxide (Merck, Darmstadt, Germany) or potassium phosphate buffer (KPB; Merck) before

they were added to the whole-blood samples. The tested endogenous metabolites included glucose (Merck), ascorbic acid (Merck), bilirubin (Sigma), and cholesterol (Sigma). All of the metabolites were dissolved in KPB solution before they were added to the whole-blood sample (UA level = 5.36 mg/dl by the reference method), except cholesterol, which was dissolved in isopropanol (J.T. Baker Chemical Co., Phillipsburg, NJ). An Artax analyzer (Via Sette Santi, Florence, Italy) was used for evaluation of the serum levels of cholesterol, ascorbic acid, and bilirubin. These samples, which actually contained high concentrations of potential interferences, were measured with the reference method (EPAC 6140) and the UASure[®].

Statistical Methods

The results were expressed as mean $\pm$ standard deviation (SD). The intra-assay CV and analysis of variance (ANOVA) were used to determine the meter precision and variability. We used Pearson's procedures to test the correlation between the UASure[®] and the Hitachi 7600. Linear regression with Deming's method (8) was used to interpret the comparison data. We determined the agreement between two instruments by plotting the difference against the standard method, and by intraclass correlation coefficient (r_i) (9,10). Agreement was considered meaningful if the lower limit of the 95% confidence interval (CI) for r_i was at least 0.75 (9). A P value less than 0.05 was considered statistically significant.

RESULTS

Imprecision

The results of the precision test and meter variability test are shown in Tables 1 and 2. The intra-assay CVs were about 5% at three different UA levels. There were no significant differences in the mean values among the different UASure[®] UA meters and the reagent strips by ANOVA.

Linearity

There was a good linear relationship between the currents of electrochemical reaction and UA concentrations in a series of diluted venous samples (range: 2.3–21.9 mg/dl). The linear regression analysis showed $R^2 = 0.9955$ ($P < 0.001$) and y -intercept = -0.0673μ A. There was also a good linearity between expected values (reference method) and actual

TABLE 1. Precision at three uric acid concentrations in whole blood

	I	II	III
<i>n</i>	20	20	20
Mean (mg/dl)	5.845	7.145	13.535
SD (mg/dl)	0.28	0.4123	0.4171
CV (%)	4.79	5.77	3.08

SD, standard deviation; CV, coefficient of variation.

TABLE 2. Comparison of inter-meter and inter-reagent strip batch difference

	Inter-meter			Inter-reagent strip batch		
	A	B	C	123	124	125
<i>n</i>	10	10	10	10	10	10
Mean (mg/dl)	10.28	10.03	10.27	10.66	10.8	11.07
SD (mg/dl)	0.29	0.38	0.25	0.68	0.79	0.58
<i>p</i> (ANOVA)		0.174			0.450	

values by UASure®. The linear regression analysis showed $R^2 = 0.9954$ ($P < 0.001$) and y -intercept = 0.1124 mg/dl (range: 3.5–18.5 mg/dl) (Fig. 3).

Analytical Recovery

The samples were tested by the UASure® in 10 replications. Analytical recovery of UA at 5 mg/dl ranged from 97.1% to 102.4% (Table 3).

Interference

The results of the interference evaluation are shown in Table 4. Only three drugs (diclofenac, glibenclamide, and methyl-dopa) and three endogenous metabolites (ascorbic acid, bilirubin, and cholesterol) interfered with the UASure® measurement in this study. When the blood cholesterol level was around 272 to 372 mg/dl, the degree of interference by blood cholesterol was –15.9 to 22%. The degree of interference by serum bilirubin was 9.9% to 23.7% while serum bilirubin level was ranging from 1.22 to 1.97 mg/dl.

Methods Comparison

The results of the UA levels ($n = 146$) were 7.23 ± 2.31 , 7.03 ± 2.00 , and 7.13 ± 1.93 mg/dl by UASure® (capillary), UASure® (venous), and Hitachi 7600, respectively. There was a good lin-

Fig. 3. Linear relationship between the expected UA concentrations (reference method) and detected values by the UASure® (10 replications) from a series of diluted venous samples. The reference method of UA measurement was the uricase method (EPAC 6140 (Eppendorf) and Uric Acid Plus 1661868 Kit (Roche)).

TABLE 3. Analytical recovery of uric acid added to whole blood

Added	Uric acid (mg/dl)			Recovery, %	CV
	Calculated	Observed ^a			
0		4.30			4.2
5	9.30	9.42		102.4	2.7
10	14.30	14.01		97.1	2.6

^aTen replicates by UASure.

ear relationship between UA levels measured by the UASure® and Hitachi 7600 in the capillary and venous samples (Fig. 4A and B). The linear regression analysis showed $R^2 = 0.6086$ and y -intercept = –1.7798 mg/dl in the capillary samples, and $R^2 = 0.7562$ and y -intercept = –1.1015 mg/dl in the venous samples (both $P < 0.0001$). While the agreement between the UASure® and the standard method was determined by plotting against the reference method (Fig. 5A and B), the differences were limited (capillary 0.10 ± 1.45 ; venous -0.09 ± 1.01 mg/dl). We also

Fig. 4. Relationship between UA concentrations obtained by the Hitachi 7600 and UASure®. The linear regression and Pearson's correlation coefficient were applied to the comparison of the Hitachi 7600 (venous blood) and the UASure® with (A) capillary blood and (B) venous blood. $S_{y/x}$ = SD of the residual.

TABLE 4. Uric acid levels measured by UASure and reference method with different spiked interferents

	Highest spiked levels with interference < 15%			Lowest spiked levels with interference > 15%		
	Spiked level (mg/dl)	UASure (mg/dl) and interference ^b	Reference method ^a (mg/dl)	Spiked level (mg/dl)	UASure (mg/dl) and interference ^b	Reference method ^a (mg/dl)
Ketoprofen	10	5.40 (+0.7%)	5.38	2.5	6.33 (+18.1%)	5.26
Diclofenac	1.25	5.64 (+5.2%)	5.33			
Indomethacin	10	5.41 (+0.1%)	5.36			
Amiloride	20	4.82 (−10.1%)	5.22			
Colchicine	10	5.33 (−0.6%)	5.29			
Atenolol	5	5.39 (+0.6%)	5.36	80	4.29 (−20.0%)	5.24
Allopurinol	10	4.96 (−7.5%)	5.13			
Glipenclamide	40	4.78 (−10.8%)	5.39			
Metformin	40	5.38 (+0.4%)	5.33			
Tolbutamind	40	5.47 (+2.1%)	5.42			
Methyl-dopa	0.1	5.78 (+7.8%)	5.39	0.25	6.57 (+22.6%)	5.28
Glucose	400	4.60 (−14.2%)	5.19	100	4.51 (−15.9%)	5.37
Cholesterol	50	5.39 (+5.6%)	5.21			
Creatinine	8	5.39 (+0.6%)	5.27			
Bilirubin	0.5	5.89 (+9.9%)	5.25	1.25	6.63 (+23.7%)	5.13
Ascorbic acid	0.5	5.82 (+8.6%)	5.31	1.25	6.34 (+18.3%)	5.20

All values were measured with 2 replicates. The original blood levels of endogenous uric acid, glucose, cholesterol, bilirubin, and ascorbic acid were 5.36, 83, 172, 0.72, and 0.68 mg/dl, respectively.

^aBy EPAC 6140.

^bInterference percentage (UASure readings vs. 5.36 mg/dl).

determined the agreement by plotting the difference against the mean (11,12) and obtained the same results (data not shown). The intraclass correlation was good for venous samples by UASure[®] ($r_I = 0.84$, 95% CI 0.82–0.90), and somewhat below the meaningful criterion (9) for capillary samples by UASure[®] ($r_I = 0.77$, 95% CI 0.69–0.83).

DISCUSSION

To determine whether this new UA meter is clinically interchangeable with the standard uricase method, we compared them in 146 volunteers. Our results indicated that venous UA concentrations measured by the UASure[®] were in agreement with those from the standard method. However, it is not rec-

Fig. 5. The agreement between the UASure[®] and Hitachi 7600 by plotting the difference against the reference method (Hitachi 7600). The y-axis (difference) indicates UASure[®] readings of (A) capillary or (B) venous samples minus paired measurements by the Hitachi 7600. The x-axis indicates the readings

of UA concentrations by the Hitachi 7600. The solid line represents the mean of difference, and the two dashed lines indicate ± 2 SD from the mean of difference. The venous blood samples measured by the UASure[®] were in good agreement with the Hitachi 7600 ($r_I = 0.864$, 95% CI 0.82–0.90).

ommended that capillary sampling replace the standard UA measurement.

The fast UASure[®] meter, which is based on a voltammetric technique, is less costly and time-consuming than methods based on colorimetry or spectrophotometry (13). The printed, two-layer, carbon-pasted electrode is cheap and disposable. In clinical practice, patients can obtain their UA data in 30 sec, saving time at the clinic or even obviating the need to return to the clinic for test results. To our knowledge, the UASure[®] for UA measurement with one drop of blood (designed for capillary sampling) is the first commercial, portable blood UA meter in the world.

While accuracy is our main concern, the primary difficulty in testing a UA meter is the absence of established criteria for defining a "good" UA meter. Therefore, the validation procedure and criteria are based on our previous studies validating other devices (7,14). To compare the performance of the UASure[®] with the standard method, we decided that repeatability, linear regression, correlation, and agreement would be used for statistical evaluation. First, how good is the precision of the UA meter? There was no difference in meter variability (all CV values were <6%) at three different blood UA levels. This indicated that UASure[®] had good precision and little meter variability because it almost reached the precision standard (2–5% by CV) of the glucose meter (15). Second, the results showed a good linear correlation between UASure[®] measurement and standard measurement (Fig. 4A and B). However, good linear correlation does not equal accuracy (11). A high degree of scatter in the linear regression method was indicated by a high SD of the residual ($S_{y/x} = 1.13$ and 1.85 in venous and capillary samples, respectively). Plotting the difference against the mean (or against the reference method) is a good statistical method for comparing one device against another (11). But there is no consensus in the literature about the criteria of this method to evaluate UA measurements. Intraclass correlation is another statistical method for evaluating agreement (9,10). A lower limit of 95% CI of r_i beyond 0.75 is considered good agreement (9). Only venous sampling by the UASure[®] passed this criterion; capillary sampling by the UASure[®] failed in this study.

To our knowledge, there have been no previous reports in the literature concerning the relationship of capillary and venous UA levels. The differences between the UASure[®] and the standard method were 0.10 ± 1.45 (capillary) and -0.09 ± 1.01 (venous) mg/dl. A paired Student's t -test showed no significant difference in UA levels between capillary and venous sampling by UASure[®] measurement. This indicated that there is little difference between mean values in the capillary and venous UA levels. So, the scattering shown in Figure 5A contributes to disagreement of capillary sampling by UASure[®]

(capillary) and Hitachi 7600. Deficient blood volume (<6 μ l, according to the manufacturer) may affect UA level determination. It is possible to make insufficient blood for testing by capillary sampling. Venous sampling, on the other hand, always provides sufficient blood for testing. Inadequate capillary sampling may be the major cause of disagreement between the capillary samplings by the UASure[®] and the standard method.

In conclusion, the UASure[®] with venous sampling is interchangeable with the standard method for UA measurement. However, the UASure[®] with capillary sampling requires further evaluation.

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