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Evaluation of an electrochemical biosensor for uric acid measurement in human whole blood samples

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ABSTRACT

Background: Uric acid measurement has become increasingly important, and electrochemically modified detection method based portable devices hold a dominant position in the market for point of care and self-monitoring of uric acid blood levels. However, there has been a lack of detailed performance evaluation of the electrochemical detection devices that are currently being used in professional health care facilities and for home self-monitoring of uric acid.
Methods: A commercially available uric acid monitoring system that is chemically modified to reduce interference was evaluated via clinical evaluation for its performance and interference as compared to a centralized laboratory instrument.
Results: Precision was within ±3.1% for 3 levels of control solutions and whole blood samples. A range from 30 to 55% was acceptable for the measurement of hematocrit levels in whole blood samples. There was no interference for the potential subtracts at their high therapeutic levels. Hemolyzed samples of up to 75 g/l showed no interference with test results obtained by the BeneCheck system, while a −45.9% bias% was obtained during testing of the same samples by a spectrophotometer. Clinical evaluation showed that >95% of tests were within ±20% bias% compared to a centralized instrument in hospitals.
Conclusion: The uric acid monitoring system was suitable for use in monitoring or screening uric acid concentration for home users or professionals.

1. Introduction

Uric acid is the end metabolism product of purine, purine being the nitrogen-containing component that occurs in nucleic acid. Uric acid is only slightly soluble in water and may precipitate out of solutions contributing to the formation of kidney stones. Uric acid measurement recently became important due to elevated levels which were observed in many patients with medical and health conditions [1–5] beyond gout [6].

In earlier days, uric acid was measured with the chemical reduction of photungstate complexes and involved a complicated process [7]. Uricase was used in specific catalysis of uric acid and enhanced the selectivity of uric acid determination [8]. Colorimetric procedures were the traditional technology for uric acid determination; either photungstate complexes or uricase catalysis to induce the chromophoric absorption change in the measurement process. Uricase methods with colorimetry or spectrophotometry are the most popular testing methods in use in clinical practice.

Electrochemistry technology was considered as a replacement for the spectrophotometer, based on the desire to reduce expensive equipment and to construct portable near patient devices [9,10].

Chemically modified screen printed electrode technology provided a new turning point for biochemical determination technology [11,12]. A non-enzymatic method, provided by chemically modified electrodes [13], was one of the most promising methods for uric acid determination, not only eliminating the problem of maintaining stability during enzyme preservation but also reducing the cost of supplying enzymes. Uric acid detection has become increasingly important for point of care and patients' self-monitoring. Currently, the majority of the portable uric acid monitoring devices on the market are mostly based on electrochemically modified technology.

According to the explanations of currently market available electrochemical uric acid monitoring systems, almost all are an application of the non-enzymatic method. Electrochemical uric acid testing methods are superior to the commercial enzymatic spectrophotometric method in several aspects: (1) a short detection time (normally <20 s for electrochemical method compared to about 10 min for spectrophotometric method); (2) no sample pretreatment step for electrochemical method;

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blood cells need to be removed for the spectrophotometric method due to interference caused by red blood cells; and (3) reagent (test strips) is stable for 18 months stored at room temperature for electrochemical method; but reagent for spectrophotometric method is only stable for 4 months and also requires refrigeration at temperatures of 2–8 °C after reconstitution. However, interference from many common medications and biological materials such as acetaminophen and ascorbic acid was the most common problem encountered when applying non-enzymatic method for uric acid measurement, due to the similarity of their chemical characteristics [10]. Although a list of materials that could potentially cause interference with test results is usually emphasized in the instruction manual of the uric acid monitoring system [14], this does not increase patient confidence during usage. Furthermore, there was not sufficient information studying the effects of interference for some devices [15].

2. Materials and methods

2.1. Materials

The BeneCheck PLUS multi-monitoring system for glucose, uric acid and total cholesterol (General Life Biotechnology) was used to evaluate the uric acid measurement function. The BeneCheck system contains test strips and a meter. The BeneCheck PLUS uric acid test strips were prepared by the General Life Biotechnology Co., Ltd using the chemically modified screen printed electrode technology to construct a two-electrode system with carbon paste as the working electrode and silver/silver chloride paste (Ecron) as the counter electrode. The working electrode surface was treated with 1.9 V for 15 s in a phosphate buffer (0.1 mol/l, pH 7.4). A passageway with a top cover from the tip of the strip to the electrodes was constructed for the strip to form a channel for capillary sample intake.

The uric acid measurement principle behind BeneCheck was based on amperometric electrochemistry. A whole blood sample is drawn by capillary action into the reaction zone of the strip. The uric acid in the whole blood is oxidized by the electrode, and a current proportional to the concentration of uric acid is detected by the meter when a fixed potential is applied across the electrodes. The current is then converted into a reading of uric acid concentration. The BeneCheck measuring range of uric acid is 30 mg/l to 200 mg/l, this range is wide enough to cover most patients. Sample volume required is 1 μl and measurement time is 15 s according to the instructions of the BeneCheck monitoring system.

BeneCheck PLUS meter used for uric acid determination is a palm size, battery-powered, light weight instrument designed for self-monitoring of capillary blood uric acid concentration.

Material used for the interference study included bilirubin, cholesterol, acetaminophen, creatinine, allopurinol, amiloride, atenolol, colchicine, diclofenac, gentisic acid, hypoxanthine, ibuprofen, metformin, tetracycline, tolazamide, tolbutamide and xanthine which were from Sigma. Glucose was from Baker. Ascorbic acid, hydrochloric acid and sodium hydroxide were from RDH while dopamine and methyl DOPA were from Aldrich. Glibenclamide, ketoprofen, L-tryptophan, sodium chloride, sodium L-lactate and sodium nitrite were from Sigma-Aldrich. Indomethacin and salicylate were from Fluka. Vacutainers with different anticoagulants including sodium heparin, sodium fluoride, sodium citrate and potassium EDTA were all from Becton Dickson.

2.2. Methods

2.2.1. Sample preparation

2.2.1.1. Uric acid stock solution preparation. Uric acid stock solution was prepared by adding uric acid powder (Sigma) into 0.08 mol/l of lithium carbonate solution (Sigma) to a concentration of 250 g/l.

2.2.1.2. Venous blood sample preparation. Venous blood samples were collected directly into vacutainer tubes containing heparin as an anticoagulant. Hematocrit of the blood sample was measured with Sysmex KX-21N automatic whole blood analyzer and the hematocrit of the sample was adjusted to 42.5 ± 0.5% by adding or removing plasma of the blood sample. The uric acid concentration of the venous blood sample was then adjusted by adding different volumes of the uric acid stock solution. The venous blood tubes were placed on a shaker for at least 30 min on gentle rotation.

2.3. Precision evaluation

Three levels of control solution with different uric acid concentrations provided by General Life Biotechnology were tested. Twenty-five replicates of each of the three level control solutions were measured by 1 m. Three different concentrations of venous blood samples were prepared for precision evaluation. Five replicates of each concentration of samples were measured by 1 m. Five meters were used for a total of 25 test results. The mean, standard deviation and the percentage of the coefficient of variation of the test results were calculated.

2.4. Hematocrit effect study

Venous blood samples with differing uric acid concentrations were prepared as previously described in the sample preparation method. According to the instructions for BeneCheck uric acid strips, acceptable hematocrit of blood samples ranges from 30% to 55% for uric acid measurement. The expected uric acid concentration for samples used in this hematocrit effect study was defined as 65 ± 10 mg/l, 100 ± 10 mg/l and or 125 ± 10 mg/l, as measured by Cobas analyzer. For each uric acid concentration, venous blood was then aliquot to micro-centrifuge tubes and adjusted to different hematocrit concentrations ranging from 30% to 55% by adding plasma or removing plasma after centrifugation. The uric acid concentration in each tube was measured with a BeneCheck monitoring system. After measurement by the BeneCheck monitoring system, the hematocrit of the venous blood sample was measured with Sysmex KX-21N automatic whole blood analyzer. Samples were also centrifuged and the uric acid concentration of the plasma was measured with Cobas C111 chemistry analyzer.

2.5. Interference study

Studies were done to evaluate the interference caused by certain substances towards the BeneCheck Plus uric acid strip test results. Three categories of substances with the potential to cause interference: endogenous substances, exogenous substances, and preservatives, were involved in the study.

Concentrations of interference material in this study were prepared following NCCLS Document EP7-A2 guideline [16] or EP7-P [17] if the information was not in the EP7-A2 guideline. According to Appendix D of EP7-A2, the recommended test concentration (common pathological value) pH is 8.0, while the normal pH range of a blood sample is 6.8–7.8. Blood samples were adjusted to a pH of 6.8 with hydrochloric acid (0.6 mol/l) and a pH of 8.0 with sodium hydroxide solution (0.05 mol/l). The interference effect was evaluated for blood samples with a pH range of 6.8 to 8.0 by BeneCheck and Cobas.

Evaluation of anticoagulants was studied using 4 different commercial available vacutainers. Drawing venous blood into a 10 ml BD vacutainer with 158 USP U of sodium heparin to capacity resulted in a sample with a heparin concentration around 1580 USP U/dl, which was used as the standard reference for the uric acid sample. Venous blood from the same blood donor was injected into other BD vacutainers containing potassium EDTA (18.0 mg), sodium fluoride (17.5 mg) or sodium citrate (0.129 mol/l). Uric acid concentration in each tube was measured by BeneCheck and the bias% to the reference sample was calculated for each sample.

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