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Calibration of glucose oxidase-based test strips for capillary blood measurement with oxygen saturated venous blood samples

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ABSTRACT

Background: Glucose oxidase biosensors are used in self-monitoring blood glucose concentrations. The capillary blood glucose quantitation requires a calibration curve. Due to the limitation in obtaining calibration curve from capillary blood, an alternate approach by using venous blood for neonatal measurement was investigated.
Methods: A signal correlation between oxygen saturated venous blood and capillary blood was derived. The hematocrit effect was studied for different glucose concentrations. The calibrated glucose strips were validated by neonatal intensive care unit (NICU) samples.
Results: A simple equation, finger blood signal = 1.39 * (oxygen saturated venous blood signal) − 31.2 was derived. The rate of change in glucose concentration due to hematocrit effect was low in lower glucose concentration samples. The BeneCheck Glucose Strips were compared with Beckman Coulter analyzer by using 52 NICU samples. More than 95% of test results were within the variation of ±10 mg/dl of bias and ±15% of bias% when glucose concentration is <75 mg/dl and ≥75 mg/dl respectively.
Conclusions: The BeneCheck Glucose Strips can be accurately calibrated with venous blood. The hematocrit effect can also be predicted. Based on this study, BeneCheck Blood Glucose Monitoring System can be suitable for neonatal glucose measurement.

1. Introduction

Electrochemical biosensors are used for measuring the glucose concentration in whole blood samples and are routinely used by diabetic patients at home and also by clinical professionals. Various enzymes have been employed in the electrochemical blood glucose monitoring systems [1]. The glucose oxidase (GOD) enzyme with an electron mediator is a commonly used method in glucose biosensors. The principle of the detection is, in general, glucose reacts with GOD and transfers two electrons to GOD. Then GOD passes the two electrons through a mediator to the electrode and generates a current. The current generated is directly proportional to the glucose concentration. However, GOD may also transfer electrons to oxygen and water forming hydrogen peroxide. This step would reduce the current generated by glucose and falsely lower the result. As such these sensors are very sensitive to the fluctuating oxygen content in the measured samples. Since the oxygen may compete with a mediator to use up the electrons from the reduced form of GOD enzyme, the reduced mediator will be low if the oxygen content of the sample is high [2,3]. Therefore, when the capillary blood samples are used for GOD biosensor strips, the strips should be calibrated with a matrix that has a similar oxygen concentration as capillary specimens to achieve an optimal performance [4].

Studies have shown that the oxygen concentration in venous blood is lower than in capillary blood. Thus, for equivalent glucose concentrations a higher current will be generated with venous blood if using GOD biosensor strips [2]. Meanwhile, the capillary/finger blood is much easier to acquire and convenient for the purpose of self-monitoring blood glucose measurement with a handheld device. However this procedure is impractical for the calibration process of such a device, as it requires a large quantity of capillary blood samples from finger lancing with different levels of glucose concentration and similar matrix property. Therefore, it is a common practice to use venous blood samples for calibration purposes. Venous blood with desired volume is much easier to acquire for preparing the samples with different glucose concentrations and also to maintain the same matrix properties such as hematocrit and other biological contents. In venous blood, the

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oxygen concentration and the partial pressure of oxygen will increase during the sample preparation and reach its saturated level within 15 min [2,3] and when the blood sample is only few microliters, it is even <1 min.

The electrochemical method based blood glucose test strips are normally fabricated with the screen printing method and have achieved good clinical performance. Most of the studies related to the screen printed electrode for glucose or other test targets demonstrated their performance either with clean aqueous solution or with venous blood samples [5,6]. Due to the possible and significant deviation between venous and capillary blood samples, it becomes prerequisite to correlate the test results from venous blood sample to capillary blood sample. In order to construct a calibration curve for glucose in capillary blood test using venous blood samples, the relationship between venous blood and capillary blood should be studied.

Hematocrit effect is the ratio of the volume occupied by packed red blood cells to the volume of the whole blood. The term hematocrit effect is used here to describe the influence of varying hematocrit concentrations on glucose measurements. Due to the differential distribution of glucose between blood cells and plasma, the effect of hematocrit on the current produced by saturated venous blood samples during the GOD reaction requires further investigation [7,8]. Furthermore, the total oxygen content may differ in different hematocrit samples after oxygen saturation.

In neonates, it has been known that the hematocrit levels may be higher (up to 60%) but their normal blood glucose concentration will be lower than normal adults [9]. For newborns, at risk for neonatal hypoglycemia [10], glucose concentration should be monitored with high frequency. Using linear regression of glucose rate of change as a function of hematocrit to the sample glucose level, the hematocrit effect can be predicted. This study involved a simple mathematical approach to correlate the signal of oxygen saturated venous blood and capillary blood measured by the glucose test strip. This can be used to compensate the anticipated signal changes between venous blood and capillary blood. The blood samples collected from NICU were tested by BeneCheck Blood Glucose Monitoring System.

2. Materials and methods

2.1. Materials

BeneCheck Blood Glucose Monitoring System (General Life Biotechnology, Taipei, Taiwan) which includes strips and a meter was used for this study. The BeneCheck Blood Glucose Test Strip consists of immobilized glucose oxidase and potassium hexacyanoferrate as a mediator [11]. When the whole blood sample is introduced to the meter, a potential of 0.33 V against a carbon reference electrode is provided by the glucose meter. This helps to oxidize the reduced form of a mediator and generates a reaction current. The amplitude of the current is proportional to the concentration of glucose in the whole blood sample.

BeneCheck Blood Glucose Test Strips were prepared by General Life Biotechnology Co., Ltd. by using screen printing method to construct a 2-electrode system with carbon paste (Ecron, MA, USA). A strip coating reagent was prepared in phosphate buffer along with glucose oxidase (Toyobo, Osaka, Japan) and potassium hexacyanoferrate (Sigma, St. Louis, MO) and passively coated on the working electrode surface. A passage from the tip of the strip to the electrode working area was constructed to form a channel for sample uptake using capillary force.

2.2. Glucose stock solution

Glucose stock solution was prepared by dissolving glucose (Sigma) in deionized water (Milli-Q, Millipore, Billerica, MA) at a concentration of 25 g/dl.

2.3. Oxygen saturated venous blood sample

The venous blood samples were collected directly in vacutainer tubes (BD, Franklin Lakes, NJ) with heparin as anticoagulant and allowed to stand overnight to reduce its glucose concentration. The hematocrit level of the blood samples was measured by Sysmex KX-21N Hematology Analyzer (Kobe, Hyogo, Japan) and it was adjusted to $42.5 \pm 0.5\%$ by adding or removing the plasma or cells from the blood samples.

The venous blood was aliquoted into 8 microcentrifuge tubes and the glucose concentration of the venous blood was adjusted to 600, 500, 400, 300, 200, 100, 75 and 50 mg/dl separately by adding respective volumes of glucose stock solution. The venous blood tubes were placed on a shaker (Biosan, Riga, Latvia) at least for 30 min with gentle rotating.

2.4. Venous blood signal and glucose concentration measurement

The glucose concentration in each tube was measured using the BeneCheck Blood Glucose Meter and Strip with its signal mode. The electrochemical signal was obtained for each tube with different glucose concentrations. After the signal was measured, the venous blood was centrifuged and the plasma was measured for glucose concentration with Cobas c111 analyzer (Roche, Switzerland).

2.5. Glucose concentration in finger blood measurement

The BeneCheck Blood Glucose Meter with electrochemical signal mode was used to measure finger blood from the patients. The glucose concentration in finger blood of the same person was also measured with Accu-Chek Advantage (Roche) and FreeStyle (Abbott, Abbott Park, IL) blood glucose monitoring systems at the same time for comparison.

2.6. Construction of calibration curve for finger blood glucose concentration

A calibration curve of the oxygen saturated venous blood was obtained by plotting the glucose concentration measured from Cobas c111 analyzer to the signal measured by BeneCheck Glucose Meter. A linear calibration curve will be:

$$Y_{vs} = a_{vs}X_{vs} + b_{vs} \tag{1}$$

where, Y_{vs} is the glucose concentration measured from oxygen saturated venous blood using Cobas c111 analyzer; X_{vs} is the oxygen saturated venous blood signal measured by BeneCheck Glucose Meter; a_{vs} is the slope of regression line; and b_{vs} is the intercept.

A calibration curve of finger blood was constructed by plotting the glucose concentration and finger blood signal.

$$Y_f = a_f X_f + b_f. \tag{2}$$

Here the f denotes the finger blood. Y_f is the average glucose concentration measured with Accu-Chek and FreeStyle glucose meters. X_f is the finger blood sample signal obtained from BeneCheck Glucose Meter, a_f is the slope of the regression line, and b_f is the intercept. Eq. (2) will be the calibration curve stored in BeneCheck Glucose Meter for concentration conversion from the measured signal of finger blood sample.

For example, when the glucose concentration is n, $Y = n$ and expressed as Y_n, then Eq. (1) will be,

$$Y_n = a_{vs}X_{vsn} + b_{vs}$$

and Eq. (2) will be,

$$Y_n = a_f X_{fn} + b_f$$

$$X_{vsn} = (Y_n - b_{vs})/a_{vs}; X_{fn} = \left(Y_n - b_f\right)/a_f$$