ORIGINAL ARTICLE

New Plasma Separation Glucose Oxidase-based Glucometer in Monitoring of Blood With Different PO$_2$ Levels

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Background: The PalmLab glucometer is a newly designed plasma separation glucose oxidase (GO)-based glucometer. Past studies have shown that the accuracy of GO-based glucometers is compromised when measurements are taken in patients with high PO$_2$ levels. We performed a two-arm study comparing the fitness of the PalmLab blood glucometer with that of a standard glucose analyzer in monitoring blood glucose levels in pediatric patients, especially when arterial partial pressure of oxygen (PO$_2$) was high.
Methods: In the first arm of the study, arterial blood samples from pediatric patients were measured by the PalmLab blood glucometer and the YSI 2302 Plus Glucose/Lactate analyzer. In the second arm of the study, venous blood samples from adult volunteers were spiked with glucose water to prepare three different levels of glucose (65, 150, and 300 mg/dL) and then oxygenated to six levels of PO$_2$ (range, 40−400 mm Hg). The biases of the PalmLab glucometer were calculated.
Results: A total of 162 samples were collected in the first arm of the study. Results of linear regression showed that the coefficient of determination (R^2) between PalmLab glucometer and standard glucose analyzer was 0.9864. Error grid analysis revealed that all the results were

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within Zone A (clinically accurate estimate zone). The biases between the two systems were low at different PO_2 levels. In the second arm of the study, the results were also unaffected by changes in PO_2.
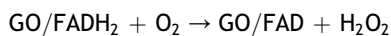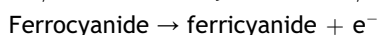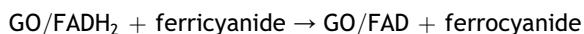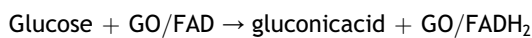
Conclusion: The PalmLab glucometer provides accurate results in samples with high PO_2 and is suitable for measuring arterial glucose levels in pediatric patients.
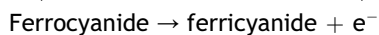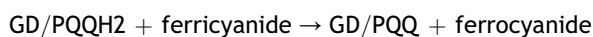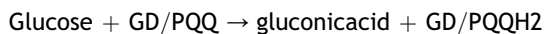
1. Introduction

Daily bedside glucose monitoring is a routine practice in pediatric intensive care units. Arterial lines are frequently inserted in this group of patients, and arterial samples can be drawn from these lines or from skin punctures for rapid glucose testing. There are several glucose monitoring systems available today, and these glucose meters can be roughly classified into glucose dehydrogenase (GD)-based system or glucose oxidase (GO)-based systems. The accuracy of traditional GO-based monitoring systems is affected by high PO_2 values, which limits their clinical use for arterial blood samples.[1,2] GO-based test strips use GO to catalyze the oxidation reaction that changes glucose into gluconic acid. The levels of glucose will be detected by the number of electrons that connects to an electrode, produced by several sequences of oxidation and reduction reactions. Oxygen competes with electron mediators (reduced flavin adenine dinucleotide) produced after the above-mentioned reactions. No electrons are produced in the oxygen side of the reaction. It, therefore, follows that lower blood glucose readings will be obtained in blood samples with higher PO_2 values. The chemical reactions measured by GO- and GD-based test strips are displayed below:

1. GD-based strips

$Glucose + GO/FAD \rightarrow gluconicacid + GO/FADH_2$

$GO/FADH_2 + ferricyanide \rightarrow GO/FAD + ferrocyanide$

$Ferrocyanide \rightarrow ferricyanide + e^-$

$GO/FADH_2 + O_2 \rightarrow GO/FAD + H_2O_2$

2. GD-based strips

$Glucose + GD/PQQ \rightarrow gluconicacid + GD/PQQH2$

$GD/PQQH2 + ferricyanide \rightarrow GD/PQQ + ferrocyanide$

$Ferrocyanide \rightarrow ferricyanide + e^-$

where FAD = flavin adenine dinucleotide; $FADH_2$ = reduced flavin adenine dinucleotide; PQQ = pyrroloquinoline quinone; PQQH2 = reduced pyrroloquinoline quinone.

The PalmLab blood glucose monitoring system is a GO-based monitoring system manufactured by Sand County Biotechnology Inc., Taiwan. It is equipped with a microporous blood filtration layer. The PalmLab glucose test strip is composed of two conductive electrodes covered with a reaction chamber in the blood testing area. The reaction chamber is composed of two layers: the microporous blood filtration layer and the reagent layer. When a drop of blood is drawn into the reactive chamber, the blood cells are blocked at microporous blood filtration layer from entering the reagent layer. Therefore, glucose content is only measured in the plasma portion of the blood sample (Figure 1).

Pre-clinical and clinical trials have shown that the PalmLab glucometer can measure glucose concentrations in the range of 10−600 mg/dL (0.6−33.3 mmol/L). It has been shown to perform well within an operating temperature range of 10−40°C in both venous and capillary samples of adults. It takes only 0.1 μL on each glucose monitoring.[3] In this two-arm study, we evaluated the accuracy of the PalmLab glucometer in measuring glucose concentrations in arterial samples with high PO_2 levels in pediatric patients in critical care units.

2. Materials and Methods

The two-arm study was performed from February 10, 2009 to February 27, 2009 at the Chunghua Christian Hospital, Taiwan.

2.1. First-arm study patient samples and methods

In the first arm, arterial samples were collected from arterial lines or arterial punctures from 104 patients in the neonatal intensive care unit (age range, 0−1 month) and from 58 patients in the pediatric intensive care unit (age range, 0−17 years). All blood samples were collected in conjunction with existing physician's orders for laboratory testing and tested immediately for hematocrits (Hct) and PO_2 using a Stat Profile Blood Gas Analyzer (Nova Biomedical, MA, USA). Glucose concentration in an aliquot of each whole blood sample was measured by the PalmLab blood glucose monitoring system and by a reference instrument, the YSI 2302 Plus Glucose/Lactate analyzer (Yellow Spring Instruments, OH, USA), an instrument that has been shown not to be affected by the blood oxygen effect.[4] The study protocol was approved by the institutional review board of the Chunghua Christian Hospital, and informed consent was obtained from the parents of each patient before blood sampling.

According to ISO 15197 guideline for *in vitro* glucose monitoring systems, 95% of individual glucose rapid test results shall fall within 15 mg/dL of the standard manufacturer's measurement procedure at glucose concentrations less than 75 mg/dL and within 20% of glucose concentrations greater than or equal to 75 mg/dL.[5] We, therefore, plotted a bias graph to show the effect of PO_2 on glucose monitoring.
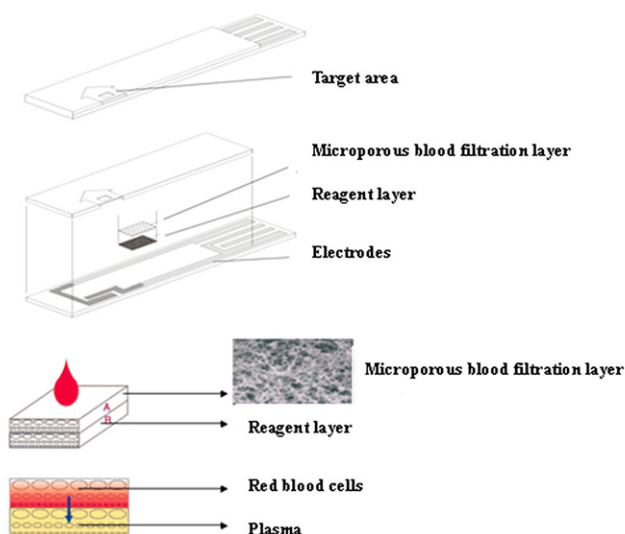
Figure 1 Structure of the multilayer element of PalmLab glucose test strip for quantifying plasma glucose from the whole blood sample.

Biases of the results from the PalmLab system were calculated using the following formulas:

$$Bias\,(mg/dL) = (YSI\ result - PalmLab\ result);$$
$$at\ glucose\ level \leq 75mg/dL$$

$$Bias\,(\%) = \{(YSI\ result - PalmLab\ result)/(YSI\ result)\}$$
$$\times 100\%;\ at\ glucose\ level > 75mg/dL$$

2.2. Second-arm study patient samples and methods

In the second arm of the study, blood samples from healthy adult volunteers who had not taken any medication before blood donation were analyzed to determine whether high blood PO_2 levels would affect the glucose readings from the PalmLab system. Samples were collected in lithium heparin Vacutainer tubes (Becton-Dickson, Rutherford, NJ, USA) and mixed well by inverting 20 times. The heparinized blood was kept at room temperature overnight for glycolysis to glucose level of 0 mg/dL. Glucose was then added to venous samples to prepare three different levels of glucose: low (65 mg/dL), medium (150 mg/dL), and high (300 mg/dL). The spiked samples with different glucose levels were then oxygenated with a Piston-type regulator and flow meter (Genstar Technologies Inc., Chino, CA, USA) to desired PO_2 levels. Six levels of oxygenated blood samples were prepared: 40, 80, 120, 240, 320, and 400 mm Hg. The oxygen content of each prepared blood sample was verified by a blood gas analyzer (Stat Profile, Nova Biomedical, Waltham, MA, USA). Blood samples with different glucose and oxygen levels were measured three times by the PalmLab glucometer. The mean values of the results from the three readings were calculated and compared with those measured by the oxygen-insensitive YSI reference instrument. The oxygen effect was evaluated

based on the mean difference (instrument bias) in glucose value between the PalmLab glucose meter and the YSI reference instrument. The biases were recalculated according to ISO 15197 guideline.

2.3. Statistical analysis

In the first arm of the study, the correlation between the PalmLab results and the reference results was analyzed using least-squares linear regression analysis. The clinical accuracy of the blood glucose monitors in estimating glucose concentration was examined using the error grid analysis proposed by Clark et al.[6,7] The glucose concentrations measured by the PalmLab system were plotted against the reference values within a grid divided into five zones.

Results in zone A are clinically accurate estimates. The values in this zone deviate from the reference by less than 20% at glucose concentrations greater than or equal to 75 mg/dL and less than 15 mg/dL when the glucose concentrations are less than 75 mg/dL. Zone B is a region of acceptable estimates in which the estimate values deviate by greater than 20% at glucose concentrations greater than or equal to 75 mg/dL. Zone C results indicate over-correcting of acceptable blood glucose levels, and Zone D results are potentially dangerous and represent failure to detect and treat glucose levels outside the desired target range. Finally, data in Zone E would lead to treatment that is the opposite of what is appropriate.[8,9]

3. Results

The characteristics of the 162 patients (104 neonates and 58 children) from whom blood samples were taken are listed in Table 1. The blood glucose concentrations ranged from 43.0 mg/dL to 458 mg/dL, and the PO_2 levels ranged from 29.5 mm Hg to 341.7 mm Hg. Linear regression analysis (Figure 2) showed good correlation (coefficient of determination, $R^2 = 0.986$) between the results obtained from the PalmLab glucometer and those obtained from the reference instrument (YSI 2302 Plus Glucose/Lactate analyzer).

The clinical accuracy was analyzed according to the methodology suggested by ISO 15197 guideline. In the 18 patients with glucose levels less than or equal to 75 mg/dL (4.2 mmol/L), we found that glucose concentrations fell within 15 mg/dL (0.83 mmol/L) of the reference range; the concentrations in 12 (66.7%) of the 18 patients fell within ± 5 mg/dL of the reference range; and the levels in 6 (33.3%) of the 18 patients were within ± 10 mg/dL of the reference range (Table 2). In the 142 patients with glucose concentrations greater than 75 mg/dL, the levels were within 20% of the reference range (Table 3).

The clinical significance of errors in glucose determinations was analyzed using the error grid analysis method proposed by Clarke et al.[6] A scatter-plot of PalmLab results (y-axis) versus YSI Stat Plus results (x-axis) was overlaid with an error grid consisting of a line of identity ($y = x$) and zones of clinical significance. Each point on the scatter-plot fell into one of these zones. The error-grid analysis showed that all measured glucose results were within the clinically

Table 1   Demographics of participants

Demographic characteristic	
No. of patients	162
PICU	104
NICU	58
Gender	
Female/male	92/70
Age (yr)	
Mean	7.8
Range	0−17.0
SD	4.8
Glucose (mg/dL)	
Mean	127.8
Range	44.2−599.0
SD	73.6
PO_2 (torr; mm Hg)	
Mean	90.9
Range	29.5−341.7
SD	36.0

PICU = pediatric intensive care unit; NICU = neonatal intensive care unit; SD = standard deviation

accurate estimate zone (Zone A). None of the data fell into inferior zones, such as Zone B or Zone C (Figure 3).

Blood gas analysis was performed at the time of blood sampling in order to analyze the possible impact that PO_2 had on glucose measurements. There was a wide variation in PO_2 levels among the 162 patients (range, 29.5−341.7 mm Hg). The bias plot for the PalmLab system (relative to the YSI reference method) shows that the test results were not significantly affected by the PO_2 in arterial blood samples (Figure 4). The biases in the group of patients with blood glucose levels less than 75 mg/dL ranged from −9.4 to 6.7 mg/dL, and those in the group of patients with glucose levels greater than 75 mg/dL ranged
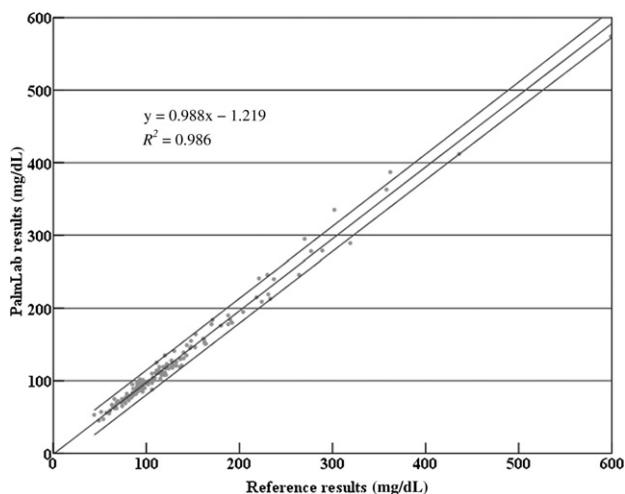
Figure 2   Pearson's correlation and simple linear regression between PalmLab results and reference results. ($n = 162$; $p < 0.001$; and 95% confidence interval of regression coefficient is 0.970−1.006).

Table 2   System accuracy results for glucose concentration $\leq$ 75 mg/dL (4.2 mmol/L)

Within ± 5 mg/dL (within ± 0.28 mmol/L)	12/18 (66.7%)
Within ±10 mg/dL (within ± 0.56 mmol/L)	18/18 (100%)
Within ±15 mg/dL (within ± 0.83 mmol/L)	18/18 (100%)

from −12.7% to 17.0%. The biases conformed to the requirements of ISO 15197 guideline.

Figure 5 shows the results of the second arm of the study. As seen in blue line, changes in PO_2 (range, 39−419 mm Hg) in blood samples with low glucose concentrations (65 mg/dL) affected the accuracy of PalmLab blood glucose monitoring system within acceptable range (bias range, −4.8 to 0.2 mg/dL). Similarly, as seen in red and green lines, changes in PO_2 (range, 40−406 mm Hg) in blood samples with moderate (150 mg/dL) and high (300 mg/dL) glucose concentrations did not affect the accuracy of the glucometer markedly. The biases in both cases were within 10% (range, −6.4% to 2.6% and −7.9% to 2.9%, respectively).

4. Discussion

Studies have shown that the accuracy of GO-based glucometers is compromised when measurements are taken in patients with high PO_2 levels.[10,11] This problem is especially relevant in neonates because fetal hemoglobin has higher O_2 affinity than adult hemoglobin. In this two-arm clinical study, we found that the results obtained from the newly designed plasma separation bedside PalmLab (GO-based) blood glucose monitoring system correlated well with those obtained from a standard laboratory blood glucose system at high PO_2 levels in neonates and pediatric patients in critical care units. Although in Figure 5 we can still find higher bias at high PO_2 samples, all test results were within the acceptable range defined by ISO 15197 guideline. At 37°C, 1 mL of plasma contains 0.00003 mL O_2/mm Hg PO_2, and each gram of hemoglobin is capable of combining with about 1.34 mL of oxygen under the same conditions. In a patient with a hemoglobin level of 13 g/dL, the plasma oxygen content is less than 2% of the total blood oxygen content.[12] Such a little amount of oxygen in the chemical reaction of a GO-based glucose monitoring system may not affect the result markedly and can explain why the PalmLab glucometer performs well when measuring glucose content in high PO_2 samples.

Some of the commercially available glucose test strips contain glucose dehydrogenase-pyrroquinoline quinone (GD-PQQ). Studies have shown that the results obtained from those types of test strips are not affected by oxygen because oxygen does not compete with electromediators for $PQQH_2$ (reduced form of pyrroloquinoline quinone), and are not

Table 3   System accuracy results for glucose concentration of greater than 75 mg/dL (4.2 mmol/L)

|	
Within ± 5%	71/142 (50.0%)
Within ± 10%	131/142 (92.3%)
Within ± 15%	141/142 (99.2%)
Within ± 20%	142/142 (100%)
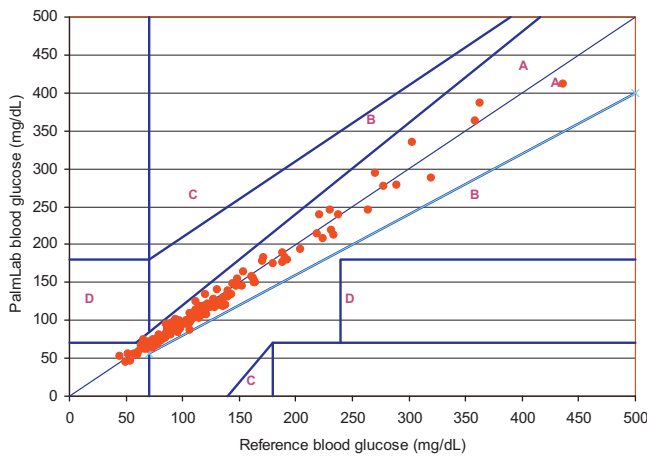
Figure 3 The error grid analysis shows that all measured glucose results are within the accurate estimate zone (Zone A).

affected by high PO_2.[13,14] However, icodextrin, a glucose polymer widely used in continuous ambulatory peritoneal dialysis (Extraneal) interferes with the accuracy of GD-PQQ-based glucose meters. Icodextrin enters the systemic circulation during peritoneal dialysis and is hydrolyzed by amylase to maltose or other oligosaccharides. GD-PQQ-based glucometers cannot distinguish maltose from glucose. Therefore, pseudohyperglycemia may lead to administration of insulin, which may lead to hypoglycemia.[15] In patients receiving maltose, icodextrin, galactose, or xylose, clinicians must avoid using GD-PQQ-based glucose meters and should only use glucose dehydrogenase nicotinamide adenine dinucleotide (GDH-NAD)-, GO-, or glucose hexokinase-based glucometers.[16,17] Galactosemia in pediatric patients can produce inaccurate readings when glucose levels are measured with GD-PQQ-based handheld point-of-care systems. Maltose-, galactose-, and xylose-containing intravenous immunoglobulin products can cause the same problem.[18]

In our study, only two patients in the pediatric intensive care unit had a PO_2 greater than 200 mm Hg in the first arm
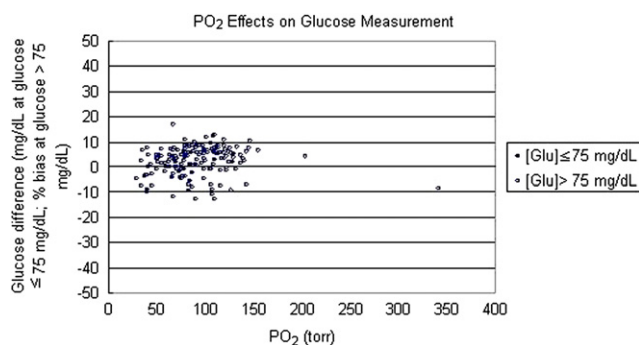
Figure 4 The plot of biases for PalmLab system (relative to the YSI reference method) at different PO_2 levels. The biases in the group of patients with blood glucose levels of less than 75 mg/dL ranged from −9.4 to 6.7 mg/dL and those in the group of patients with glucose levels of greater than 75 mg/dL ranged from −12.7% to 17.0%. The biases conform to the requirements of ISO 15197 guideline.
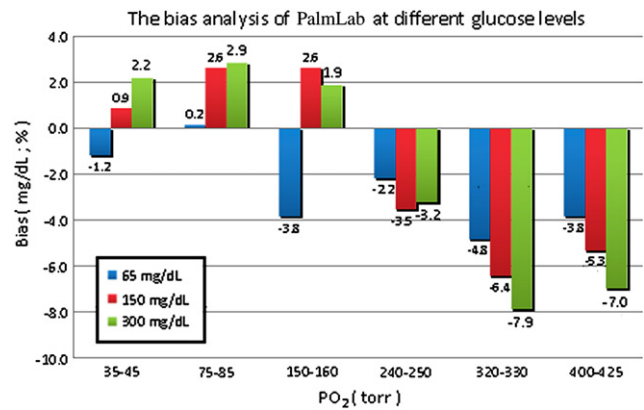
Figure 5 Results of low (65 mg/dL), medium (150 mg/dL), and high (300 mg/dL) glucose levels are shown in blue, red, and green lines, respectively. Changes in PO_2 (35−425 mm Hg) did not affect the accuracy of results; all the biases were within 10 mg/dL when glucose level is less than or equal to 75 mg/dL and within 10% when glucose level is greater than 75 mg/dL. (For interpretation of the references to color in this figure legend, the reader is referred to the online version of this article).

of the study, although the two rapid glucose test results conformed with the ISO 15197 guideline definition. To improve this limitation, we oxygenated blood samples from adult volunteers in the second arm of the study, and showed that the PalmLab provided accurate results in samples with a PO_2 of 400 mm Hg.

Another benefit of the PalmLab system is that it directly measures plasma glucose levels. Most handheld glucometers measure whole blood glucose levels and present plasma-equivalent values using a conversion factor of 1.11 (plasma = whole blood × 1.11). In polycythemic patients, however, this conversion factor can lead to false-positive low glucose level readings.[19−21] Therefore it is conceivable that neonates who have relatively high hematocrits will have more accurate rapid test results when blood separation test strips are used. Further study is required to check the clinical accuracy of the PalmLab system in polycythemic newborns.

In conclusion, the results obtained from the plasma separation PalmLab blood glucose monitoring system correlate well with those obtained from the YSI Stat Plus system in arterial blood samples with high PO_2 levels in pediatric patients in critical care units.

Acknowledgments

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