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Accuracy of portable human glucose meter (Accu-chek® Performa) for blood glucose measurement in newborn piglets

Morakot Nuntapaitoon^{1,3*} Sujin Sirisawadi² Sariya Asawakarn² Padet Tummaruk^{1,3}

Abstract

Blood glucose concentration is a significant postnatal survival indicator in newborn piglets. The standard procedure is difficult to perform under field conditions and requires a long period of time for processing. Portable glucose meters have been invented to yield a faster result and utilize a small blood sample. The present study aims to compare glucose concentration measured using a portable human glucose meter (Accu-chek® Performa) with the standard protocol. A total of 200 blood samples were collected from the umbilical cord of newborn piglets and were measured using the portable human glucose meter immediately after birth. Serum was extracted from the blood sample and was also analysed using the glucose oxidase method. Both methods were compared for each sample and were analysed for agreement between the two methods. The average blood glucose concentrations in neonatal piglets were 49.1 ± 23.7 and 49.2 ± 23.7 mg/dl, with the 95% confidence intervals between 45.8 and 52.4 mg/dl and 45.9 and 52.9 mg/dl for the portable blood glucose meter and the glucose oxidase technique, respectively. The blood glucose concentrations determined using the two methods highly correlated ($r = 0.839$, $P < 0.001$). The statistical test used to determine the agreement between the two methods indicated that 96.5% of the difference in blood glucose concentration between the two methods was within the mean \pm (2 \times standard deviation), indicating good agreement between the two methods. In conclusion, a portable human glucose meter can be used to determine blood glucose concentration in neonatal piglets.

Keywords: glucose concentration, piglet, portable human glucose meter, umbilical blood

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Introduction

Blood glucose concentration is a significant postnatal survival indicator for neonatal piglets (Baxter et al., 2008; Nuntapaitoon et al., 2018). In humans, hypoglycaemia has been detected in infants with sepsis and hypoxia-ischaemia, and in premature neonates (Beardsall, 2010). Neonatal hyperglycaemia is associated with mortality and morbidity, including retinopathy of prematurity and intraventricular haemorrhage (Beardsall, 2010). In pigs, a high blood glucose concentration in neonatal piglets is associated with pre-weaning mortality and growth performance (Panzardi et al., 2013; Nuntapaitoon et al., 2018). A greater risk of mortality up to 3 or 7 days after birth has been associated with low (24–30 mg/dl) and high (45–162 mg/dl) blood glucose concentrations (Panzardi et al., 2013). Low blood glucose levels have been linked with the nutritional status of the piglet during the birth process. On the other hand, a high blood glucose concentration has been associated with the risk of suffering in the piglet during parturition (Herpin et al., 1996). Adrenaline is released into the blood stream in low viability piglets, being essential for glycogenolysis and increasing glucose levels (Herpin et al., 1996; Alonso-Spilsbury et al., 2005). Piglets with low viability have a delay between birth and first suckling and decreased colostrum consumption, leading to low immune status (Quesnel et al., 2012). Moreover, the blood glucose concentration in newborn piglets is related to growth performance before weaning. Nuntapaitoon et al. (2018) found that piglets with a blood glucose concentration of ≤ 24 mg/dl had a worse growth performance than piglets with a blood glucose concentration of > 24 mg/dl in the suckling period.

Automated machines (e.g., ADVIA 560®, Siemens, Germany) are commonly used to determine blood glucose in many standard laboratories. The machines usually use the glucose oxidase and hexokinase methods as a gold standard (Sacks et al., 2002). Although the glucose oxidase method is highly accurate, the processing time is relatively long. Glycolysis decreases glucose concentrations in whole blood at a rate of 5% to 7% per hour (Sacks et al., 2002). Therefore, a delay in transporting the specimen to the laboratory can decrease glucose levels and cause diagnostic error. A portable blood glucose meter is an alternative to reduce falsely low glucose levels. Furthermore, it can yield fast result, using a very small amount of blood and is a noninvasive method.

A number of portable glucose meters using the strip test have been widely used in humans, e.g., Accu-chek® Performa (Accu-Chek® Performa, Roche, Mannheim, Germany), Sure-step™ Onetouch (LifeScan, Inc., Milpitas, CA, USA), PCx (Abbott/Medisense, Bedford, MA, USA) and Contour®TS (Bayer HealthCare, Tarrytown, NY, USA). These glucose meters are based on an electrochemical system, detecting electrical currents after the blood sample reacts chemically with the strip. The enzyme on the test strip, a mutant variant of quinoprotein glucose dehydrogenase (Mut, Q-GDH) from *Acinetobacter calcoaceticus*, recombinant in *E. coli*, converts the glucose in the blood sample to gluconolactone. This reaction creates a harmless DC electrical current that is

interpreted to give the blood glucose level. In the standard glucose oxidase test, the glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The resulting hydrogen peroxide reacts under catalysis of peroxidase with phenol and 4-aminophenazone to produce a red-violet quinoneimine dye. The immediate and accurate diagnosis of blood glucose levels allows better neonatal care and enhances farrowing management. Glucometer performance relative to laboratory clinical analysis has been assessed for humans (Brunner et al., 1998), dogs and cats (Suvarnavibhaja et al., 2014; Mori et al., 2016), pet ferrets (Summa et al., 2014) and wildlife (Bennett et al., 2017). However, the accuracy of portable glucose meters has never been validated in swine. The present study thus aimed to compare blood glucose concentrations in neonatal piglets under field conditions using a portable glucose meter and the standard glucose oxidase method.

Materials and Methods

Animals: The experiment followed the guidelines documented in The Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes edited by the National Research Council of Thailand, and was approved by the Institutional Animal Care and Use Committee (IACUC) in accordance with the university regulations and policies governing the care and use of experimental animals (Approval number 1431063). The present study was carried out on a commercial swine herd in Thailand. Blood samples for glucose analysis were collected from 200 newborn piglets immediately after birth (i.e., before first suckling). A blood sample (a mixture of venous and arterial blood) was collected from the umbilical cord and kept in 9 ml serum separated clot activator tubes (Vacuette®, Greiner Bio-One GmbH, Kremsmünster, Austria). An aliquot of 0.6 μ l of whole blood sample was used to determine blood glucose concentration in a portable human glucometer (Accu-Chek® Performa, Roche, Mannheim, Germany). Another whole blood sample was centrifuged at 2,000 \times g for 10 min at room temperature. Serum was separated within 30 min after collecting. All samples were kept at -20°C for analysis of blood glucose concentration using the glucose oxidase test as the standard protocol.

Glucose measurement: Blood glucose was measured in whole blood with a portable human glucose meter (Accu-chek® Performa) and serum glucose was measured using the glucose oxidase method with glucose liquicolor® (HUMAN Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany) as the standard protocol. The limitation measurement of the two methods is shown in Table 1.

Portable human glucose meter: Accu-chek® Performa (Roche Diagnostics, Germany) was used in this study. A test strip is placed in the meter and the user verifies that the code number on the display matches the code number on the test strip container. When the test strip and blood drop symbols appear on the display, a drop of blood is placed on the tip of the test strip. The test requires approximately 0.6 μ l of whole blood, reports a

whole blood glucose result in 5 secs, and has a measuring range of 10–600 mg/dl.

Glucose oxidase test: The serum glucose concentration was determined after enzymatic oxidation in the presence of glucose oxidase using the glucose liquicolor® test kit (HUMAN Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany). Briefly, 1,000 µl enzyme reagent and 10 µl of the serum sample (standard reagent) were added to a plastic tube and incubated at room temperature for

10 min. All samples were analysed in duplicate. The absorbance was measured at 500 nm using a Genesys™ 20 spectrophotometer (Thermospectonic®, NY, USA) within 60 min of incubation. The glucose concentration (mg/dl) in the samples was quantified by calculating the absorbance divided by the standard value generated in parallel from the samples and multiplied by 100 according to the test kit instructions. The intra- and inter-assays coefficient of variation were 5.5% and 2.4%, respectively.

Table 1 Measurement range of the two test techniques

Method	Minimum (mg/dl)	Maximum (mg/dl)
Accu-chek® Performa	10	600
Glucose oxidase method	2	750

Statistical analyses: Statistical analysis was performed using the SAS software (SAS Institute Inc., Cary, NC, USA). Descriptive statistics were carried out using the MEANS procedure. The accuracy of the portable human glucose meter was evaluated by comparing the glucose concentration obtained from the portable human glucose meter with the level from the standard method. A linear regression model of the values acquired from the standard method and portable human glucose meter was constructed and the correlation coefficient (r) was calculated. Calculation of mean differences between the values of the portable human glucose meter and reference method was performed by Bland-Altman plot (Bland and Altman, 1986). The blood glucose concentration was classified into 2 groups (≤ 24 and > 24 mg/dl) according to the growth performance of the piglets (Nuntapaitoon et al., 2018). The comparison of blood glucose concentration for the two techniques between ≤ 24 and > 24 mg/dl of blood glucose concentration level was compared using Student's t test. $P < 0.05$ was regarded as statistically significant.

Results

The average blood glucose concentrations in neonatal piglets were 49.1 ± 23.7 and 49.2 ± 23.7 mg/dl, with the 95% confidence intervals between 45.8 and 52.4 mg/dl and 45.9 and 52.9 mg/dl for the portable blood glucose meter and the glucose oxidase technique, respectively. The range of blood glucose concentrations and coefficient of variation are shown in Table 2. The blood glucose concentrations were plotted as a linear regression model that is presented in

Fig. 1. There was a significant correlation between the portable human glucose meter and the glucose oxidase method ($r = 0.839$, $P < 0.001$).

The statistical test for determining the agreement between the two methods (Bland and Altman 1986) indicated that 96.5% of the difference in blood glucose concentration between the two methods was within the mean $\pm (2 \times \text{standard deviation})$, indicating good agreement between the two methods. Fig. 2 illustrates the difference between the blood glucose concentration obtained from the portable human glucose meter and glucose oxidase method plotted against the means of blood glucose concentration. This scatter plot diagram was constructed following a method previously described by Bland and Altman (1986). On average, the difference between the portable human glucose meter and glucose oxidase method was 0.03 ± 13.5 mg/dl. The difference between the portable human glucose meter and glucose oxidase method ranged between -54 and 47 mg/dl.

The comparison of blood glucose concentration for the two techniques between ≤ 24 and > 24 mg/dl of blood glucose concentration level is presented in Table 3. It was found that mean blood glucose levels measured by Accu-chek® Performa method were 18.7 ± 4.7 and 52.1 ± 22.7 mg/dl in ≤ 24 and > 24 mg/dl of blood glucose concentration level classes, respectively ($P < 0.001$). In addition, mean blood glucose levels by the glucose oxidase method were 28.9 ± 16.0 and 51.2 ± 23.5 mg/dl in ≤ 24 and > 24 mg/dl of blood glucose concentration level classes, respectively ($P < 0.001$).

Table 2 Descriptive statistics of blood glucose concentration for the two test techniques (n = 200)

Method	Range (mg/dl)	Mean \pm SD	%CV	95% CI
Accu-chek® Performa	10 – 166	49.1 ± 23.7	48.3	45.8 - 52.4
Glucose oxidase method	3 - 167	49.2 ± 23.7	48.3	45.9 - 52.9

SD: standard deviation

%CV: coefficient of variation

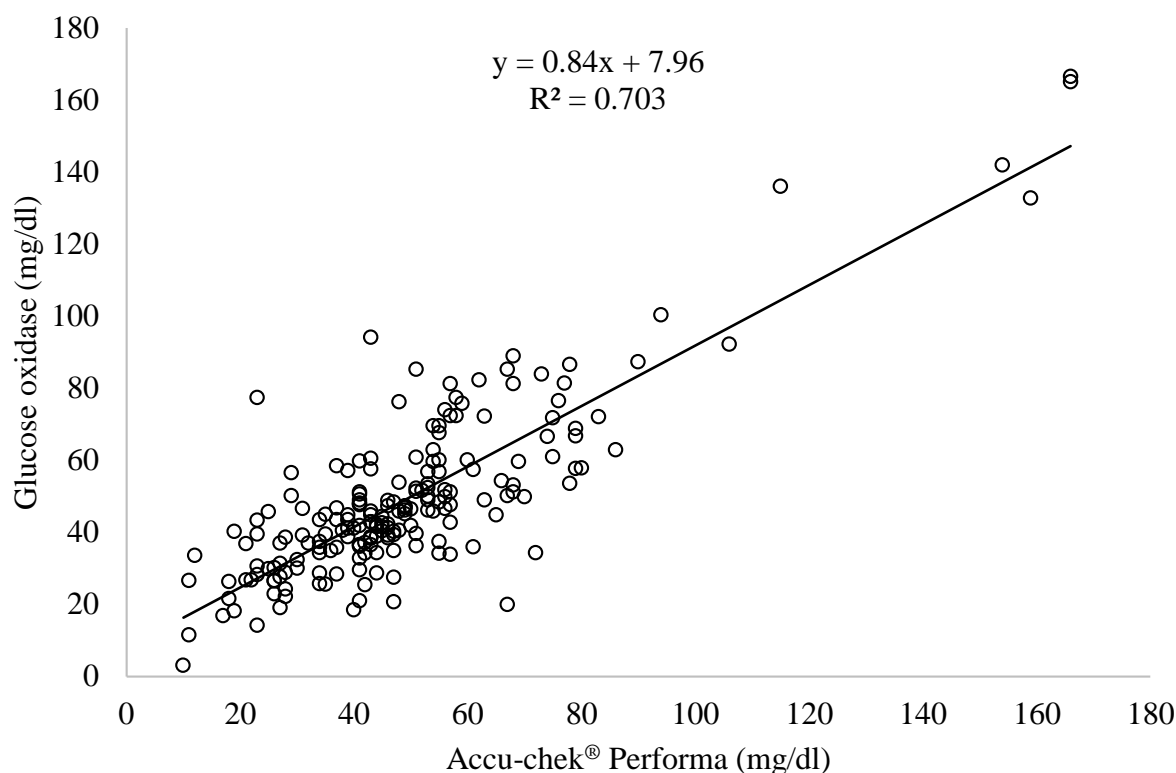
95% CI: 95% confidence interval

Table 3 Comparison of blood glucose levels between ≤ 24 (n = 18) and > 24 (n = 182) mg/dl of blood glucose concentration for the two test techniques

Method	Accu-chek® Performa		Glucose oxidase method	
	≤ 24 mg/dl	> 24 mg/dl	≤ 24 mg/dl	> 24 mg/dl
Means \pm SD (mg/dl)	18.7 \pm 4.7	52.1 \pm 22.7	28.9 \pm 16.0	51.2 \pm 23.5
95% CI	16.4 - 21.1	48.8 - 55.5	20.9 - 36.8	47.7 - 54.6
Difference between means	33.4 \pm 21.8		33.4 \pm 22.9	
P-value	< 0.001		< 0.001	

SD: standard deviation

95% CI: 95% confidence interval

**Figure 1** Scatter plot correlating concentration of glucose between Accu-chek® Performa samples (x-axis) and glucose oxidase method (y-axis)

Discussion

The aim of this study was to compare the blood glucose concentrations measured by the portable human glucose meter (Accu-chek® Performa) with those obtained using the standard glucose oxidase method. The glucose peroxidase method was the standard of glucose measurement in a number of previous studies (Williams, 1997; Ferri et al., 2011; Suvarnavibhaja et al., 2014; Mehta and Munde, 2017). The present study is the first report on determining the accuracy of blood glucose concentrations in newborn piglets measured using a portable human glucose meter. Blood glucose concentration in newborn piglets is very important for their survival within the few minutes after birth (Panzardi et al., 2013). Both low and high blood glucose levels were related to physiology of newborn piglets. In a previous study, newborn piglets with poor nutritional status had a low blood glucose concentration and hypoxic piglets had a high blood

glucose level during the birth process (Herpin et al., 1996). In addition, blood cells continue to metabolize glucose after the specimen has been obtained, which decreases the accuracy of blood glucose measurement. High accuracy and fast measurement are necessary for birth intervention and to help the piglet in the first day of life. The present study measured the blood glucose concentration in newborn piglets immediately after birth in a commercial swine herd. The portable human glucose meter can be used to determine blood glucose concentration from the umbilical cord blood vessel of the piglets within a few minutes after birth. Moreover, the instrument is available in general drug stores, is easy to use and gives reproducible readings.

Previous studies have demonstrated the accuracy and utility of portable human glucose meters in humans and animals (Lyon et al., 2010; López et al., 2012; Cheng et al., 2013; Suvarnavibhaja et al., 2014; Bennett et al., 2017). López et al. (2012) demonstrated

that a glucose meter (StatStrip) is accurate enough for glucose monitoring in intensive care unit (ICU) patients. In dogs, Suvarnavibhaja et al. (2014) found that Sure-step™ was more accurate than Accu-chek® for determining blood glucose concentration. In cows, the Precision Xceed® hand-held meter was used to determine blood glucose concentration and beta-hydroxybutyric acid concentration. This meter was

highly sensitive and specific for the detection of pregnancy toxemia and ketosis (Panousis et al., 2011; Voyvoda and Erdogen, 2010). In addition, a portable human glucose meter was used in seals by Bennett et al. (2017) for health monitoring and physiological assessment. All studies mentioned above agreed that this method was easy and convenient under field conditions.

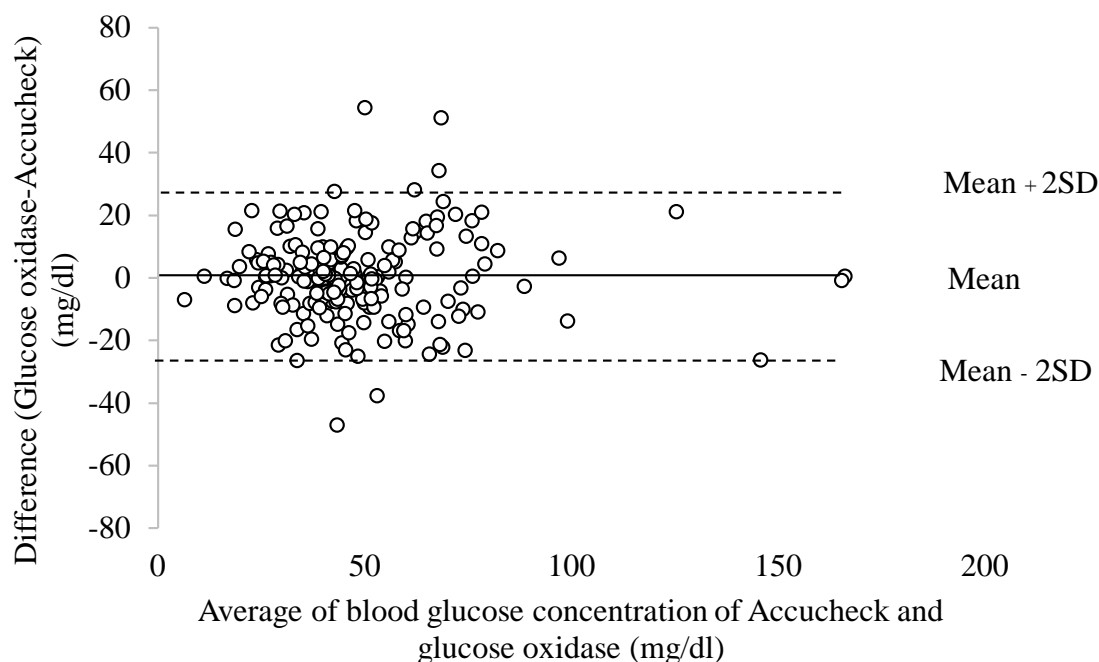


Figure 2 Bland-Altman difference plot of the difference between Accu-chek® Performa samples and glucose oxidase method plotted against average (Accu-chek® Performa samples + glucose oxidase method/2)

The correlation between the portable human glucose meter and the standard method was statistically significant. The portable human glucose meter correlation coefficient was over 0.8, and in the Bland-Altman difference plot, no significant differences were detected between the two mean differences ($P > 0.05$). This particular finding means that the portable human glucose meter provides acceptable and comparable glucose values. Moreover, 96.5% of samples were within the mean \pm ($2 \times$ standard deviation) in the present study. This indicates strong agreement between the two methods. However, factors influencing the accuracy of the glucose measurement include the species of animal, the organ from which blood samples were collected, blood chemical level, temperature and humidity, and medicines (King et al., 1995; Erbach et al., 2016). Temperature and moisture changes influenced glucometer and test strips (King et al., 1995); a previous study found that too low (8°C) and too high (36°C) temperatures changes glucose value. The actual levels erroneously read in the hypoglycaemic range in the cold temperatures. The read result was higher than the actual result. On the other hand, the read result was lower than actual result in hot temperatures. Moreover, Erbach et al. (2016) observed the factors influencing the accuracy of the glucose measurement include user, meter-inherent accuracy, environment,

physiology and medicine. Blood glucose meter measurement may be compromised by use of deteriorated test strips, which may result from inappropriate storage, mechanical stress, or usage after the expiry date. Physiological factors have been observed to potentially impact the performance (i.e., peripheral blood perfusion, hematocrit, oxygen, triglycerides, bilirubin, and uric acid). Studies have found that glucometer readings from blood of suckling animals were compromised (Heinemann, 2010; Lindholm and Altimiras, 2016; Bennett et al., 2017) because young animals have high levels of circulating fat (Schweigert, 1993). In addition, the haematocrit value influenced the blood glucose level measured by some portable human meters (Lyon et al., 2010). In conclusion, the portable human glucose meter is accurate and can be used under field conditions to determine the blood glucose concentration of neonatal piglets.

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