Evaluation of three different hand-held devices for measuring glucose concentration in capillary blood obtained by a minimal invasive lancet technique in dairy cows

Diplomarbeit

Veterinärmedizinische Universität Wien

vorgelegt von

Benedikt Mair

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**Betreuer**
Dr.med.vet. Michael Iwersen  
Klinik für Wiederkäuer, Abteilung Bestandsbetreuung für Wiederkäuer

**Gutachter**
Univ.-Prof. Dr.med.vet. Thomas Wittek  
Klinik für Wiederkäuer
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1. Introduction

Glucose is an essential nutrient for all higher organisms. Certain vitally important cells and tissues like the brain, erythrocytes and the mammary gland are dependent on the energy provided by glucose. Hence, it is important that the concentration of glucose in the blood is kept at a physiological level (Aschenbach et al. 2010). Ruminants and particularly dairy cows have a special position with respect to the glucose metabolism, because of the massive demand of their mammary gland for glucose at the onset of lactation and the permanent alteration between pregnancy and lactation. Furthermore, most of the glucose is generated by gluconeogenesis using volatile glucogenic precursors (e.g. propionate, lactate, alanine, valerate and isobutyrate) (De Koster and Opsomer 2013).

For most tissues, the supply and utilization of nutrients is regulated by hormones. Also in Ruminants insulin is a key player in the energy metabolism, influencing various metabolic processes in muscles, adipose tissue, liver and the mammary gland (Sasaki 2002). Ruminants are reported to suffer from ‘insulin resistance’ (IR) at the end of gestation and during early lactation (Petterson et al. 1993, Bell and Bauman 1997, Hayirli 2006;). The phenomenon of IR is characterized by a less than normal biological response to a physiological concentration of insulin, as a result of decreased sensitivity of the tissues, a decreased responsiveness to insulin or a combination of both (Kahn 1978).

Insulin resistance at the end of gestation and at early lactation are considered as physiologic mechanism to ensure the glucose and hence energy supply for the gravid uterus and lactating mammary gland. However, IR can transgress into a pathologic state, leading to ketosis for instance with chronically increased concentrations of β-hydroxybutyric acid (BHBA) in cows (De Koster and Opsomer 2013). From an aetiological point of view two different types of ketosis exist: First the hypoglycaemic-hypoinsulinaemic form, ‘Type I’-ketosis, which occurs three to six weeks after calving in high-yielding cows where the energy demand in the udder exceeds the capacity of gluconeogenesis. Second, the hyperglycaemic-hyperinsulinaemic form, ‘Type II’-ketosis exists, which occurs earlier in lactation as a consequence of overfeeding the animals in the dry period (Holtenius and Holtenius 1996). Besides the
measurement of the BHBA concentration, analysing the glucose concentration in blood is reported as helpful instrument for diagnosis and especially differentiation between these two types of ketosis (Voyvoda and Erdogan 2010), although Herdt (2000) described the glucose concentration as an insensitive measure of the energy status of an animal.

Monitoring metabolic parameters requires multiple blood sampling, which is usually done by repeated venepuncture in cattle. This procedure requires handling or restraining of the animal, which may result in stress-induced hyperglycemia (Wiedmeyer et al. 2005). In other species, new techniques have been introduced in which small amounts of blood are rapidly analysed for glucose concentrations using electronic hand-held devices (Wess and Reusch 2000, Zeugswetter et al. 2010, Zeugswetter and Karlovitz 2014). Applying these minimal invasive techniques to obtain capillary blood in dairy cattle might be beneficial in generating reliable and valid glucose concentrations.

The objective of this study was to test whether capillary blood can be used to determine the glucose concentrations in dairy cows using minimal invasive techniques. A second objective was to evaluate test characteristics of three commercially available hand-held devices for determination of blood glucose concentrations. Therefore a capillary blood drop, obtained from the skin of the exterior vulva, was tested with three different hand-held devices and compared with reference samples analyzed in a laboratory.
2. Material and methods

2.1 Study design

The study was approved by the institutional ethics committee of the University of Veterinary Medicine, Vienna (04/12/97/2013; date of approval 17 December 2013) according to the Good Scientific Practice guidelines and the national authority according to § 26 of Law for Animal Experiments [Tierversuchsgesetz 2012 – (TVG 2012); BMWF GZ 68.205/0007-II/3b/2014] as well as by the Slovakian Regional Veterinary Food Administration (428/2014) and was conducted between March and April 2014. A commercial dairy farm in Slovakia with approximately 2,700 Holstein-Friesian cows and additional youngstock was chosen as study site. The dairy herd was kept in free stall barns with rubber mats on concrete floors. Before calving the cows were moved in a free stall barn with straw beddings. A TMR was fed twice per day and pushed up frequently. The average energy corrected milk yield (based on 4.0 % butterfat and 3.4 % protein) was 9,165 kg in 2013.

In total, 240 dairy cows were enrolled in the study. Animals of all lactations within 2 weeks ante-partum up to 4 weeks post-partum were eligible for enrollment.

To obtain capillary blood, the skin of the exterior vulva was dry-cleaned and punctured with one of three different available lancets, in randomly order. The lancets used in the study [Microtainer Contact-Activated Lancet (MT, Becton-Dickinson), SafetyLancets special (SL, MED TRUST Handelsges.m.b.H.), MiniCollect Safety Lancets (MC, Greiner Bio-One International AG)] have a penetration depth of 2 mm with differing blade widths between 0.8 (SL) to 1.5 mm (MT and MC). If the volume of the obtained blood drop was insufficient for determination of the glucose concentration, another puncture was made. The quantity of punctures was recorded on a pre-assembled data capture form. Three different electronic hand-held meters [FreeStyle Precision, (FSP, Abbott GmbH & Co. KG), GlucoMen LX Plus, (GLX, A. Menarini GmbH), WellionVet Gluco Calea, (WGC, Med Trust Handelsges.m.b.H.)] and the associated electrochemical test strips [FreeStyle Precision blood glucose, (Abbott Diabetes Care), GlucoMen LX Sensor, (A. Menarini GmbH), WellionVet Gluco Calea test strips, (Med Trust Handelsges.m.b.H.)] were used in the study to determine
the blood glucose concentration. The WGC was already validated for use in dogs, cats and horses by using preprogrammed species-specific chips, offered with each batch of test strips. Because of best possible match in terms of red blood cell parameters, the chip initially designed for use in cats was chosen for measurements in this study.

After the application of the blood on the sensor of the test strips, an electrochemical reaction starts as follows: Glucose is oxidized to gluconolactone using glucose dehydrogenase as a catalyst. Simultaneously NAD$^+$ is reduced to NADH, which is re-oxidized by an electrochemical mediator. The reduced mediator is re-oxidized by transferring electrons to the electrode surface. The resulting current is directly proportional to the glucose concentration in the blood sample. According to the manufacturer’s instructions the devices work steadily at temperatures between + 4 °C and + 40 °C.

The sensors of the three hand-held devices were directly dipped onto the surface of the capillary blood drop. After 5 seconds each, the blood glucose concentrations were presented on the display of the devices. After this procedure, an additional blood sample was drawn from a coccygeal vessel using a blood-collection tube system (Vacuette, Greiner Bio-One GmbH) consisting of a Sodium Fluorid vacuum tube (Vacuette, FX Sodium Fluoride, Greiner Bio-One GmbH) and a 20-gauge needle (Vacuette 0.9 x 38 mm, Greiner Bio-One GmbH). Blood obtained from the coccygeal vessel was tested as well with all three devices as previously described. Approximately 2 h after collection, the coccygeal blood samples were centrifuged at 2,200 x g, at a temperature of 18 °C for 5 min. (Eppendorf Centrifuge 5804, Eppendorf AG). Supernatant plasma was split into two aliquots in microtubes of 2 ml each (Microtube, Sarstedt), as reference and back-up sample and were stored at - 18 °C until further analyses. The reference sample was analyzed at the Central Clinical Pathology Unit (CCPU) of the University of Veterinary Medicine, Vienna, and was considered as the gold standard in the present study. Plasma was analyzed with an automated wet chemistry analyzer Cobas 6000/c501 (Roche Diagnostics GmbH, Vienna, Austria) using a colorimetric hexokinase method. Hexokinase catalyzes the phosphorylation of glucose to glucose-6-phosphate and adenosine-diphosphate by ATP. This reaction is coupled with an NADP colorimetric indicator system.
To evaluate the intra-assay variability of the analyses performed at the CCPU, 20 aliquot samples taken from one cow were randomly distributed between the samples to be analyzed. Furthermore, intra- and inter-assay coefficients of variations (CV) were calculated for each specific hand-held device. For this, three blood samples with different glucose concentrations based on FSP measurements with low (30 mg/dL), medium (56 mg/dL) and high (70 mg/dL) glucose concentrations were tested ten times with one device (intra-assay) and additionally with ten different devices of the same type (inter-assay).
2.2 Statistical analysis

The data were analyzed using SPSS statistics for Windows (Version 20.0; IBM Deutschland GmbH) and BiAS for Windows (Version 10.06; Epsilon-Verlag). Data were tested for normal distribution using the Kolmogorov-Smirnov-Test. For each tested hand-held device, descriptive parameters were calculated for the glucose concentrations analyzed in capillary and coccygeal blood. Additionally, the Pearson correlation coefficients were calculated between the gold standard and the glucose concentrations measured with each specific device in capillary and coccygeal blood.

A Passing-Bablok regression (Passing and Bablok 1983) was performed to compare the glucose concentration in the gold standard with the concentrations measured with the three hand-held devices in capillary and coccygeal blood. For this, the slopes and the intercepts of the regression lines and their 95% confidence intervals were determined. The intercept \(a\) reflects the constant differences and the slope \(b\) the proportional differences between the two methods. If the confidence interval for the intercept includes the value 0, no constant difference between the two methods exists. If the interval for the slope includes the value 1, no proportional difference occurs. If neither a constant nor a proportional difference could be observed, both methods can be used interchangeably (Bilić-Zulle 2011). In addition, the agreement between the gold standard and the hand-held meters were graphically depicted using the method as reported by Bland and Altman (1986).

Based on the glucose concentrations analyzed at the CCPU, samples were classified as hypo- (< 40 mg/dL), normo- (40-60 mg/dL) or hyperglycemic (> 60 mg/dL). According to these classifications sensitivities (Se) and specificities (Sp) for each hand-held device to detect hypo- and hyperglycemia were calculated. To determine optimized thresholds to identify hypo- and hyperglycemia using the hand-held meters, Receiver Operating Characteristics (ROC) analyses were performed. The closer the resulting graph of the ROC analysis is to the left upper angle of the coordinate system, the greater is the accuracy of the test (Swets 1988). The resulting area under the ROC curve (AUC) is a measure of the discriminatory power of a test to identify animals as normoglycemic and hypo- or hyperglycemic, respectively. An AUC of 1 represents a perfect test; an AUC of 0.5 and below represents a worthless test.
3. Results

The mean glucose concentration of the 20 samples taken from one cow to evaluate the intra-assay variability of measurements at the CCPU was 44.45 ± 0.83 mmol/L, resulting in a coefficient of variation (CV) of 1.86%.

In total, 240 Holstein-Friesian cows were tested. Thirty four (14.2%) of the animals were in first lactation, 97 (40.4%) in second lactation, and 109 (45.4%) in third or higher lactation. All animals were sampled between 21 days ante-partum and 29 days post-partum (median 7 days in milk; 25% Percentile 3 and 75% Percentile 13), resulting in 199 (83%) samples from lactating cows and 41 (17%) samples from dry cows.

The gold standard samples comprised two samples (0.8%) classified as hypoglycemic (glucose < 40 mg/dL), 149 samples (62.2%) as normoglycemic (glucose between 40 to 60 mg/dL) and 89 samples (37.1%) as hyperglycemic (glucose > 60 mg/dL). All lancets used in the study were eligible for capillary blood specimen collection, and only small numerical differences could be observed in the total number of incisions needed. Capillary blood could be obtained with first incision in 94% (n = 75) using the SL, 96% (n = 77) using the MT and in 96% (n = 77) using the MC lancet. An additional second (and third) incision was necessary for the MT in three (two) cases, using the SL in two (zero) and the MC in three (zero) cases. Measurements of four (1.7%) capillary blood samples using the GLX device resulted in an “E4”-failure. According to the manufacturer’s instructions this error message indicates a “damaged sensor, incorrect application or quantity of the blood sample”. Additionally, an “E3”-failure was shown by the FSP in two (0.8%) measurements of capillary blood, indicating “a test error or glucose concentrations below the detection limit”. These six measurements were repeated.

Descriptive parameters for the analyzed glucose concentrations are presented in Table 1. The Pearson correlation coefficients between the glucose concentrations analyzed in capillary blood using the hand-held meters and the gold standard were 73.3% for the FSP, 80.5% for the GLX and 41.2% for the WGC (P < 0.01, for all three devices). Using coccygeal blood,
the corresponding correlation coefficients were 86.6% for the FSP, 78.8% for the GLX and 50.5% for the WGC ($P < 0.01$, for all three devices).

**Table 1.** Descriptive statistics of the glucose concentrations analyzed in coccygeal and capillary blood using three different hand-held devices as well as in plasma analyzed at the laboratory (gold standard).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Plasma(^1)</th>
<th>Capillary blood</th>
<th>Coccygeal blood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Laboratory</td>
<td>FSP(^2)</td>
<td>GLX(^3)</td>
</tr>
<tr>
<td>Number of samples (n)</td>
<td>240</td>
<td>240</td>
<td>240</td>
</tr>
<tr>
<td>Mean (mg/dL)</td>
<td>58.17</td>
<td>39.56</td>
<td>46.96</td>
</tr>
<tr>
<td>SD (mg/dL)</td>
<td>10.48</td>
<td>11.65</td>
<td>10.75</td>
</tr>
<tr>
<td>Median (mg/dL)</td>
<td>58.00</td>
<td>38.00</td>
<td>46.00</td>
</tr>
<tr>
<td>Interquartile range (mg/dL)</td>
<td>12.50</td>
<td>18.00</td>
<td>16.00</td>
</tr>
</tbody>
</table>

\(^1\) obtained from coccygeal blood (gold standard)
\(^2\) FSP: FreeStyle Precision, (Abbott GmbH & Co. KG, Wiesbaden, Germany)
\(^3\) GLX: GlucoMen LX Plus (A. Menarini GmbH, Vienna Austria)
\(^4\) WGC: WellionVet Gluco Calea (Med Trust Handelsges.m.b.H., Marz, Austria)

The differences between the glucose concentrations analyzed in plasma at the laboratory and the concentrations measured with the hand-held devices either in whole blood obtained from a coccygeal vessel or in capillary blood are presented in Fig. 1. The glucose concentrations analyzed with the FSP and the GLX in the capillary blood as well as in the coccygeal blood resulted in smaller glucose concentrations compared with the laboratory results in plasma. Measuring the glucose concentrations using the WGC overestimated the laboratory results.
Figure 1. Differences between the glucose concentrations in plasma analyzed at the laboratory (gold standard) and the concentrations measured with three different hand-held devices in either in whole blood obtained from a tail vessel or in capillary blood. The black line inside each box represents the 50th percentile (median); the bottom and top of the boxes show the 25th and 75th percentiles. The ends of the whiskers mark the smallest and highest statistical values; outliers are labelled as o and *

Bland-Altman plots (Fig. 2) between the gold standard and coccygeal blood demonstrated a negative bias of $-4.89 \pm 6.31$ mg/dL for the FSP and $-9.98 \pm 6.67$ mg/dL for the GLX, whereas a positive bias for the WGC of $+24.64 \pm 15.19$ mg/dL was determined. Using capillary blood for testing, biases of $-18.8 \pm 7.96$ mg/dL for the FSP, $-11.2 \pm 6.42$ mg/dL for the GLX and $+20.82 \pm 15.41$ mg/dL for the WGC were detected (Table 2, Fig. 2).
Figure 2. Differences in glucose concentrations measured with three different electronic hand-held devices in coccygeal (left) or capillary (right) blood and the gold standard against their mean. The solid line in the middle represents the mean; the solid upper and lower lines represent the mean ± 2 SD.
To compare the specific results of the hand-held devices with the gold standard, a Passing-Bablok regression was performed (Table 2). Using the GLX for measurements in coccygeal blood, the confidence interval for the slope includes the value 1, but the confidence interval for the intercept did not include 0. Hence, no proportional but a constant difference was detected. Using the FSP and the WGC in coccygeal blood, however, a proportional and a constant difference exist. Similar findings for measurements using the three hand-held devices in capillary blood were detected.

Table 2. Differences between the glucose concentrations analyzed by three different hand-held devices and the laboratory results using the Bland-Altman analysis method and Passing-Bablok regression analysis.

<table>
<thead>
<tr>
<th>Device</th>
<th>Capillary blood</th>
<th>Coccygeal blood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Passing-Bablok</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Slope (b)</td>
<td>CI 95 for b</td>
</tr>
<tr>
<td>FSP</td>
<td>0.73</td>
<td>0.67-0.80</td>
</tr>
<tr>
<td>GLX</td>
<td>0.82</td>
<td>0.75-0.88</td>
</tr>
<tr>
<td>WGC</td>
<td>0.41</td>
<td>0.34-0.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSP</td>
<td>0.72</td>
<td>0.66-0.78</td>
</tr>
<tr>
<td>GLX</td>
<td>0.88</td>
<td>0.80-1.00</td>
</tr>
<tr>
<td>WGC</td>
<td>0.40</td>
<td>0.34-0.47</td>
</tr>
</tbody>
</table>

1 FSP: FreeStyle Precision (Abbott GmbH & Co. KG, Wiesbaden, Germany),
2 CI 95: 95 % confidence interval

Receiver Operating Characteristics (ROC) analyses were intended to calculate the optimal thresholds to differentiate between normo- and hyperglycemia using the electronic hand-held
devices (Table 3, Fig. 3). Because only two reference samples were classified as hypoglycemic, no reliable ROC analyses for those samples were possible. Hence, the results are not presented. For identifying hyperglycemic animals with the FSP using coccygeal and capillary blood, threshold of 59 mg/dL (Se = 0.75, Cl95 = 0.65 to 0.84; Sp = 0.91, Cl95 = 0.86 to 0.95) and 43 mg/dL (Se = 0.76, Cl95 = 0.66 to 0.85; Sp = 0.84, Cl95 = 0.77 to 0.90) were determined. Corresponding thresholds for the GLX were 49 mg/dL for coccygeal blood (Se = 0.85, Cl95 = 0.76 to 0.92; Sp = 0.70, Cl95 = 0.62 to 0.77) and 49 mg/dL for capillary blood, too (Se = 0.92, Cl95 = 0.85 to 0.97; Sp = 0.85, Cl95 = 0.79 to 0.91). For the WGC greater corresponding thresholds of 94 mg/dL analyzing coccygeal blood (Se = 0.55, Cl95 = 0.44 to 0.66; Sp = 0.85, Cl95 = 0.78 to 0.90) and 95 mg/dL for capillary blood (Se = 0.39, Cl95 = 0.29 to 0.50; Sp = 0.92, Cl95 = 0.87 to 0.96) were determined. Corresponding AUCs and Youden-Indices for all devices are presented in Table 3.
Figure 3. Receiver operating characteristics (ROC) analyses for three different hand-held devices for diagnosis of hyperketonemia either in tail vessel or capillary blood, based on serum laboratory glucose concentration in plasma > 60 mg/dL as threshold for hyperketonemia.

The calculated AUCs for measurements in capillary and coccygeal blood indicate that the GLX (93 % and 85 %) and the FSP (87 % and 92 %) were more eligible in determining hyperglycemia than the WGC (71 % and 70 %). With a calculated AUC of 93 % and a
Youden-Index of 78 % the GLX was the most capable device to detect hyperglycemia in capillary blood. Sensitivities and specificities using the optimized thresholds for all three devices are shown in Table 3. Identifying hyperglycemia in capillary blood, Se was greatest for the GLX (93 %), however Sp was greatest for the WGC (92 %).

**Table 3.** Corresponding thresholds and performance of three hand-held meters for detection of hyperglycaemia.

<table>
<thead>
<tr>
<th>Device¹</th>
<th>Capillary blood</th>
<th>Coccygeal blood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Optimized device threshold² [mg/dL]</td>
<td>AUC³ [% (CI95)]</td>
</tr>
<tr>
<td>FSP</td>
<td>43</td>
<td>87.4 (82.5-91.3)</td>
</tr>
<tr>
<td>GLX</td>
<td>49</td>
<td>93.4 (89.5-96.2)</td>
</tr>
<tr>
<td>WGC</td>
<td>95</td>
<td>70.5 (64.3-76.3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Device¹</th>
<th>Coccygeal blood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Optimized device threshold³ [mg/dL]</td>
</tr>
<tr>
<td>FSP</td>
<td>59</td>
</tr>
<tr>
<td>GLX</td>
<td>49</td>
</tr>
<tr>
<td>WGC</td>
<td>94</td>
</tr>
</tbody>
</table>

¹ FSP: FreeStyle Precision (Abbott GmbH & Co. KG, Wiesbaden, Germany), GLX: GlucoMen LX Plus (A. Menarini GmbH, Vienna Austria), WGC: WellionVet Gluco Calea (Med Trust Handelsges.m.b.H, Marz, Austria);
² based on Receiver Operating Characteristics (ROC) analyses;
³ AUC: area under the receiver operating characteristics (ROC) curve;
⁴ CI95: 95% confidence interval;
⁵ Se: sensitivity;
⁶ Sp: specificity.
The calculated inter- and intra-assay CV for analyzing the glucose concentration using the different devices are presented in Table 4. The average inter- and intra-assay CVs were acceptable for the FSP with 4.2 % and 5.2 %, but less satisfying for the GLX with 19.9 % and 14.4 %, and for the WGC with 12.6 % and 15.7 %.

**Table 4. Intra-assay and Inter-assay Coefficients of Variation for hand-held meters FreeStyle Precision (FSP), GlucoMen LX Plus (GLX) and WellionVet Gluco Calea (WGC) within low, medium and high glucose concentrations**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Glucose concentration¹</th>
<th>FSP³</th>
<th>GLX³</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Medium</td>
<td>High</td>
</tr>
<tr>
<td><strong>Intra-assay</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean [mg/dL]</td>
<td>26.80</td>
<td>46.60</td>
<td>63.50</td>
</tr>
<tr>
<td>SD [mg/dL]</td>
<td>1.99</td>
<td>2.07</td>
<td>2.27</td>
</tr>
<tr>
<td>CV² [%]</td>
<td>7.43</td>
<td>4.44</td>
<td>3.57</td>
</tr>
<tr>
<td><strong>Inter-assay</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean [mg/dL]</td>
<td>26.40</td>
<td>48.40</td>
<td>66.10</td>
</tr>
<tr>
<td>SD [mg/dL]</td>
<td>1.58</td>
<td>1.65</td>
<td>2.18</td>
</tr>
<tr>
<td>CV² [%]</td>
<td>5.98</td>
<td>3.41</td>
<td>3.30</td>
</tr>
</tbody>
</table>

¹ Glucose concentration levels: Low, Medium, High
³ FSP: FreeStyle Precision, GLX: GlucoMen LX Plus, WGC: WellionVet Gluco Calea
<table>
<thead>
<tr>
<th></th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intra-assay</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean [mg/dL]</td>
<td>109.50</td>
<td>122.20</td>
<td>119.60</td>
<td>117.10</td>
</tr>
<tr>
<td>SD [mg/dL]</td>
<td>19.70</td>
<td>20.20</td>
<td>15.20</td>
<td>18.37</td>
</tr>
<tr>
<td>CV² [%]</td>
<td>17.99</td>
<td>16.53</td>
<td>12.71</td>
<td>15.74</td>
</tr>
<tr>
<td><strong>Inter-assay</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean [mg/dL]</td>
<td>93.4</td>
<td>113.3</td>
<td>114.3</td>
<td>107</td>
</tr>
<tr>
<td>SD [mg/dL]</td>
<td>14.39</td>
<td>16.53</td>
<td>8.94</td>
<td>13.29</td>
</tr>
<tr>
<td>CV² [%]</td>
<td>15.41</td>
<td>14.59</td>
<td>7.82</td>
<td>12.61</td>
</tr>
</tbody>
</table>

1 Based on measurements using FreeStyle Precision (low = 30 mg/dL; medium = 56 mg/dL; high = 70 mg/dL);
2 Coefficient of variation = SD×100/mean;
3 FSP = FreeStyle Precision, (Abbott GmbH & Co. KG, Wiesbaden, Germany);
4 GLX = GlucoMen LX Plus (A. Menarini GmbH, Vienna Austria);
5 WGC = WellionVet Gluco Calea (Med Trust Handelges.m.b.H, Marz, Austria).
4. Discussion

Oikonomou et al. (2008) reported a significant influence of the glucose concentration on reproductive performance, as glucose is essential as major energy source for ovarian cyclicity (Rabiee et al. 1999). Additionally, a study by Galvão et al. (2010) has shown that cows suffering from uterine infections within the first three weeks after parturition had a decreased polymorphonuclear neutrophils (PMN) glycogen concentration and hence, a decrease in PMN functions. It was reported that the PMN glycogen concentration is positively associated with the blood glucose concentration. Furthermore, Garverick et al. (2013) found greater blood glucose concentration in cows that conceived at first insemination in comparison with unsuccessfully bred cows, and hypothesized that blood glucose concentration within the first three weeks after parturition can be used as predictor for the likelihood of pregnancy after first insemination.

To our knowledge, this is the first study evaluating the suitability of the three hand-held meters to determine the glucose concentration in capillary blood in dairy cows. Using a minimal invasive technique to obtain capillary blood, as already used in small animal medicine (Wess and Reusch 2000, Zeugswetter et al. 2010, Zeugswetter and Karlovitz 2014), could be useful for monitoring the energy metabolism in dairy cows. Additionally, measurements of the glucose concentrations in cows already suffering from hyperketonemia might be beneficial to gain further insights in the type of existing ketosis (Voyvoda and Erdogan 2010) in order to optimize the animals’ treatment. For these purposes, a reliable and accurate test system is essential.

Based on laboratory thresholds, the number of plasma samples (n = 2, 0.8 %) classified as hypoglycaemic was low. This was not expected because several cows were sampled in the early lactation where a negative energy balance is expected. It can be speculated if these results support the hypothesis of a physiological insulin-resistance in dairy cows (Holtenius and Holtenius 1996). Hence, further studies are planned to investigate the usefulness of population based clinical decision limits to detect a hypoglycaemic condition in cows during the transition period.
Whereas the FSP and the GLX were already validated for BHBA measurements to diagnose subclinical ketosis in dairy cows (Iwersen et al. 2009, 2013), the WGC has never been used for studies in bovines. The suitability of different hand-held devices to analyze glucose concentrations in blood obtained from the jugular vein or a coccygeal vessel in dairy cows were already evaluated by others (Roeder et al. 1996, Voyvoda and Erdogan 2010, Wittrock et al. 2013). For the FreeStyle Precision Xtra device (Abbott Diabetes Care Inc.), a previous version of the FSP, Wittrock et al. (2013) determined a Pearson correlation coefficient between coccygeal whole blood samples and the corresponding plasma glucose concentration analysed at a laboratory of 95% with a mean difference of -0.03 ± 1.96 mmol/L (equivalent to -0.54 ± 35 mg/dL). Voyvoda and Erdogan (2010) determined a correlation coefficient between the glucose concentration in jugular whole blood analyzed with the Optium Xceed device (Abbott Diabetes Care) and its corresponding laboratory plasma concentration of 63% with a mean difference of 0.27 mmol/L (4.86 mg/dL). Evaluating the One Touch II (Lifescan Inc) Roeder et al. (1996) reported a correlation coefficient of 94% with a mean difference of 12.95 mg/dL for samples obtained either from the jugular vein in calves, or by coccygeal venepuncture in cows and their plasma glucose concentrations, analysed at a laboratory. The results of our study, using the FSP and the GLX devices for analysing the glucose concentration in coccygeal blood showed similar deviations from the laboratory results as reported by Roeder et al. (1996) and Voyvoda and Erdogan (2010). A lower deviation, however, was found by Wittrock et al. (2013). The reason for this finding remains speculative, but might be caused by the repeated measurements of 81 cows resulting in 709 blood samples analysed in the study by Wittrock et al. (2013).

The highest deviation with a mean difference of approximately +21 mg/dL for measurements in capillary and +25 mg/dL in coccygeal blood compared with the gold standard was found for the WGC. A reason for this finding might be that a chip validated for cats was used in this study. Hence, the development of a cow-specific chip is recommended for this device before further studies in cattle should be conducted.

The determined average inter- and intra-assay coefficients of variation for the FSP (4.2 % and 5.2 %) were below 15 % as requested by the European Medicines Agency (EMEA, 2011) for on-site tests. The intra-assay CV of the GLX (14.4 %) and the inter-assay CV of the WGC
(12.6 \%) were within the upper limit of the requested range, whereas the inter-assay CV of the GLX (19.9 \%) and the intra-assay CV of the WGC (15.7 \%) exceeded the requested threshold in the bovine species.

The determined correlation coefficients for the glucose concentrations analyzed in capillary blood and the gold standard were high, but not excellent for the FSP (73.3 \%) and the GLX (80.5 \%), whereas the correlation for the WGC was poor (41.2 \%). The corresponding results for coccygeal blood were approximately 13 percentage points higher for the FSP, 9 percentage points higher for the WGC, but 3 percentage points lower for the GLX. A possible explanation for the poorer performance of these two devices for measurements in capillary blood might be that sometimes it was necessary to squeeze the skin of the vulva to obtain an adequate amount of blood. Squeezing the skin might cause the excretion of tissue fluid, which mixes up with the blood drop and thereby influences the glucose concentration. This, however, does not explain fewer variations in correlation coefficients for the GLX. Another possible impact on the results might be the amount of blood applied on the test strips. According to the manufacturer of the WGC, the test strip must remain sufficiently immersed into the blood after the acoustic signal of the device occurs. It should be noticed that this signal indicates the start of the measurement with the WGC, whereas for other devices this signal indicates the end of the measurement procedure.

Bland-Altman plots revealed a negative bias for the FSP and the GLX and a positive bias for the WGC. With thresholds optimized by ROC analysis, the GLX showed the best performance to detect hyperglycemia using capillary blood as test substrate. The results for the GLX were even better in the capillary blood than in the coccygeal blood. Remarkable lower sensitivities were found for WGC compared with the other devices. The Youden-Index presented in Table 3 confirms that the GLX is probably the most capable device to analyze glucose concentrations in capillary blood. Glucose concentrations determined by the FSP, however, should be interpreted with care, whereas the WGC with the current settings is unsuitable.

This study provides data that measuring glucose in dairy cows is influenced by the test system and to some extent to the site of sampling. Additional field studies are required to confirm the
hypothesized beneficial effects of monitoring the glucose concentration as predictor of the animal’s metabolic status (Voyvoda and Erdogan 2010), immune competence (Oikonomou et al. 2008, Galvao et al. 2010) or reproductive performance (Garverick et al. 2013). Furthermore the validity of clinical decision limits to address hyper- or hypoglycemic statuses, especially for cows during the transition period, has to be assessed in further clinical studies.

The objective of this study was to test whether capillary blood can be used to determine the glucose concentration in dairy cows using three different electronic hand-held devices. Good test characteristics for determining hyperglycemia by an on-site test were found for the GLX and moderate for the FSP. Hence, these devices are eligible for use in veterinary practice. With the current settings, the WGC was not suitable for determination of glucose concentrations neither in capillary nor in coccygeal blood. For this device, the development of a cattle-specific chip is recommended. Capillary blood obtained by minimal invasive puncture of the skin of the exterior vulva seems suitable for analyzing glucose concentrations.
5. Summary

The objective of this study was to evaluate the suitability of capillary blood for blood glucose measurement in dairy cows using the hand-held devices FreeStyle Precision (FSP, Abbott), GlucoMen LX Plus (GLX, A. Menarini) and the WellionVet Gluco Calea, (WGC, Med Trust). In total, 240 capillary blood samples were obtained from dry and fresh lactating Holstein-Friesian cows. Blood was collected from the skin of the exterior vulva by using a lancet. For method comparison, additional blood samples were taken from a coccygeal vessel and analyzed in a laboratory. Glucose concentrations measured by a standard laboratory method were defined as the gold standard.

The Pearson correlation coefficients between the glucose concentrations analyzed in capillary blood with the devices and the gold standard were 73% for the FSP, 81% for the GLX and 41% for the WGC. Bland-Altman plots showed biases of -18.8 mg/dL for the FSP, -11.2 mg/dL for the GLX and +20.82 mg/dL for the WGC. The optimized threshold determined by a Receiver Operating Characteristics analysis to detect hyperglycemia using the FSP was 43 mg/dL with a sensitivity (Se) and specificity (Sp) of 76% and 80%. Using the GLX and WGC optimized thresholds were 49 mg/dL (Se = 92%, Sp = 85%) and 95 mg/dL (Se = 39%, Sp = 92%).

The results of this study demonstrate good performance characteristics for the GLX and moderate for the FSP to detect hyperglycemia in dairy cows using capillary blood. With the current settings, the WGC was not suitable for determination of glucose concentrations.
6. Zusammenfassung


Der Pearson Korrelations-Koeffizient zwischen der im Kapillarblut mit den Schnelltestgeräten gemessenen Glukosekonzentration und dem Goldstandard betrug 73 % für das FSP, 81 % für das GLX und 41 % für das WGC. Die Bland-Altman Diagramme zeigten eine mittlere Abweichung von -18.8 mg/dL für das FSP, -11.2 mg/dL für das GLX und +20.82 mg/dL für das WGC. Mittels einer Receiver Operating Characteristics (ROC) Analyse wurden die Grenzwerte zur Bestimmung einer Hyperglyämie bestimmt. Für das FSP wurde ein Schwellenwert von 43 mg/dL mit einer Sensitivität (Se) und Spezifität (Sp) von 76 % bzw. 80 % ermittelt. Für das GLX und WGC wurden Schwellenwerte von 49 mg/dL (Se = 92 %, Sp = 85 %) bzw. 95 mg/dL (Se = 39 %, Sp = 92 %) ermittelt.

### List of abbreviations

- **IR**: insulin resistance
- **MT**: Microtainer Contact-Activated Lancet
- **SL**: SafetyLancets special
- **MC**: MiniCollect Safety Lancets
- **FSP**: FreeStyle Precision
- **GLX**: GlucoMen LX Plus
- **WGC**: WellionVet Gluco Calea
- **CCPU**: Central Clinical Pathology Unit
- **CV**: coefficients of variations
- **Se**: sensitivities
- **Sp**: specificities
- **ROC**: Receiver Operating Characteristics
- **AUC**: area under the curve
- **PMN**: polymorphonuclear neutrophils
References


Garverick HA, Harris MN, Vogel-Bluel R, Sampson JD, Bader J, Lamerson WR, Spain JN, Lucy MC, Youngquist RS. 2013. Concentrations of nonesterified fatty acids and

Hayirli A. 2006. The role of exogenous insulin in the complex of hepatic lipidosis and ketosis associated with insulin resistance phenomenon in postpartum dairy cattle. Veterinary Research Communications, 30:749-774.


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**Table 4:** Intra-assay and Inter-assay Coefficients of Variation for hand-held meters FreeStyle Precision (FSP), GlucoMen LX Plus (GLX) and WellionVet Gluco Calea (WGC) within low, medium and high glucose concentrations
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Figure 1: Differences between the glucose concentrations in plasma analyzed at the laboratory (gold standard) and the concentrations measured with three different hand-held devices in either in whole blood obtained from a tail vessel or in capillary blood. The black line inside each box represents the 50th percentile (median); the bottom and top of the boxes show the 25th and 75th percentiles. The ends of the whiskers mark the smallest and highest statistical values; outliers are labelled as o and *.

Figure 2: Differences in glucose concentrations measured with three different electronic hand-held devices in coccygeal (top) or capillary (bottom) blood and the gold standard against their mean. The solid line in the middle represents the mean; the solid upper and lower lines represent the mean ± 2 SD.

Figure 3: Receiver operating characteristics (ROC) analyses for three different hand-held devices for diagnosis of hyperketonemia either in tail vessel or capillary blood, based on serum laboratory glucose concentration in plasma > 60 mg/dL as threshold for hyperketonemia.