Suitability of capillary blood obtained by minimal invasive techniques to detect subclinical ketosis in dairy cows

Diplomarbeit
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1. Introduction

Subclinical ketosis (SCK) is defined as metabolic disorder with an increased ketone body concentration in the absence of clinical symptoms of ketosis (Andersson 1988, Duffield et al. 1998, Rollin et al. 2010). Commonly used thresholds to define SCK are BHBA concentrations in blood of 1.2 and 1.4 mmol/L in the first and second week of lactation, respectively (Geishauser et al. 1998, Duffield et al. 2009). The occurrence of SCK in dairy herds in the periparturient period, caused by a negative energy balance, represents an important challenge for dairy farmers. Several studies have shown that SCK is associated with an increased risk for the occurrence of secondary diseases such as clinical ketosis, displaced abomasum, metritis, mastitis and lameness (Geishauser et al. 1998, Duffield et al. 2009, Suthar et al. 2013). Additionally, a decreased milk yield (Dohoo and Martin 1984, Duffield et al. 1997) and impaired reproductive performance (Walsh et al. 2007, Chapinal et al. 2012) are associated with the occurrence of SCK. Based on BHBA concentrations in blood of ≥ 1.2 mmol/L, Suthar et al. (2013) reported an overall prevalence of SCK for 10 European countries of 21.8%, ranging from 11.2 to 36.6% within 2 weeks after calving. Reported prevalence for North American dairy herds ranged from 8.9% to 43.2% within the first 2 months of lactation (Dohoo and Martin 1984, Geishauser et al. 1998, McArt et al. 2012). Considering the abovementioned aspects, monitoring of dairy herds for SCK is reported to be an appropriate measure for disease prevention and improvement of stock management efficiency in dairy farming (Cook et al. 2006). Determination of BHBA concentrations in serum or plasma with standard laboratory methods was defined as gold standard for diagnosing of SCK (Duffield et al. 1998). This method, however, is inconvenient for a broader surveillance because of its costs in terms of time and money. The possible delay in treatment of animals suffering from SCK, because of shipping and analyzing a blood sample at an external laboratory might have a negative impact on animal welfare as well.

Within the last two decades, several point of care tests have been developed and were evaluated for dairy cows to detect ketones in urine (Carrier et al. 2004), milk (Geishauser et al. 1998, Geishauser et al. 2000, Carrier et al. 2004) and whole blood (Iwersen et al. 2009, Iwersen et al. 2013, Mahrt et al. 2014). In summary, study results indicated superiority of cowside ketone testing in whole blood over most methods based on urine or milk testing (Geishauser et al. 1998, Geishauser et al. 2000, Carrier et al. 2004, Iwersen et al. 2009, Iwersen et al. 2013).
To our knowledge, only venous or arterial blood samples or both have been evaluated as specimen categories for monitoring of ketosis using electronic hand-held devices, yet. A disadvantage of this testing method is its more invasive sampling technique compared to milk and urine based systems. Additionally, in many countries (e.g. Germany, Switzerland and the Netherlands) national legislation prohibits conventional blood sampling by lay-persons (e.g. farmers). Capillary blood might be an alternative, as sampling is considered to be less invasive and easier to achieve compared to the conventional blood sampling procedures. The permission of obtaining capillary blood by the farmer using a minimal invasive technique for diagnostic purposes is already on consideration by the authorities in Austria, for instance.

The objective of this study was to test whether capillary blood obtained from the skin of the exterior vulva by using a minimal invasive lancet technique is suitable to detect SCK in dairy cows. For this purpose a capillary blood drop was tested with 3 electronic hand-held devices that were already evaluated for monitoring of ketosis in dairy cows (Iwersen et al. 2013, Mahrt et al., 2014).
2. Materials and methods

2.1 Study design

The study was approved by the institutional ethics committee of the University of Veterinary Medicine, Vienna and the national authority according to § 26 of the Law for Animal Experiments, Tierversuchsgesetz 2012 – TVG 2012 (GZ 68.205/0007-II/3b/2014) as well as by the Slovakian Regional Veterinary Food Administration (428/2014). The study was conducted in March and April 2014 on a Slovakian dairy farm, keeping approximately 2,700 Holstein-Friesian cows and additional youngstock. Cows were housed in free-stall barns with high bed cubicles. Rubber mats with dried slurry separator material were used as cubicle bedding. The average energy corrected milk yield (based on 4.0% butterfat and 3.4% protein) was 9,165 kg in 2013.

A sample size calculation (type I error α=0.05, type II error β=0.2) was performed to detect a maximum irrelevant difference in the BHBA concentration of 0.1 mmol/L between the methods evaluated in the study, resulting in 216 animals needed. To compensate for potential data losses due to necessary exclusions because of pre-analytical or analytical problems 240 animals were enrolled in this study. For this, primi- and multiparous cows between 2 weeks ante partum up to 4 weeks post partum were used preferentially in the study because of their increased risk of developing a SCK in this period (LeBlanc 2010).

Three electronic hand held devices [FreeStyle Precision (FSP, Abbott GmbH & Co. KG, Wiesbaden, Germany), GlucoMen LX Plus (GLX, A. Menarini GmbH, Vienna, Austria), NovaVet (NOV, Nova Biomedical, Waltham, USA)] were used to analyze the BHBA concentration in capillary blood as well as in a whole blood sample obtained from a coccygeal vessel. To obtain capillary blood, 3 different types of disposable lancets [Microtainer Contact-Activated Lancet (MT, Becton-Dickinson, Franklin Lakes, USA), SafetyLancets special (SL, Med Trust Handelsgesm.b.H., Marz, Austria), MiniCollect Safety Lancets (MC, Greiner Bio-One International AG, Kremsmünster, Austria)] were used. For sampling procedures, the skin of the exterior vulva was cleaned with a paper towel, disinfected and then punctured using a minimal invasive lancet to obtain a single blood drop. The penetration depth was 2 mm for all types of lancets, with blade widths differing between 0.8 mm (SL) to 1.5 mm (MT and MC). If the obtained blood volume was insufficient for an accurate measurement with all 3 electronic hand-held devices, the bleeding was enforced by softly squeezing the skin of the
exterior vulva. If this still was unsuccessful, another puncture approx. 1 cm lateral from the first incision was performed.

The animals identification number, the date of sampling, the number of required punctures for each specific lancet and the analyzed results of all three hand-held devices for capillary and coccygeal blood were recorded onto a data capture form. The type of lancet which had to be used was pre-assigned on this data capture form, generated by using the random function in Microsoft Excel (version 6.1.760, Microsoft Cooperation, Redmond, WA).

The BHBA concentration of the capillary blood drop was immediately analyzed using all 3 electronic devices in randomly order. After inserting the test strips into the hand-held devices, the front edge of the strips were dipped directly onto the drop of blood. The operating principle was similar for all 3 devices: A chemical reaction within the test strips oxidizes the BHBA in the blood sample to acetoacetate in the presence of the enzyme BHBA dehydrogenase, with the concomitant reduction of NAD⁺ to NADH. The NADH is reoxidized to NAD⁺ by a redox mediator. This chemical reaction releases electrons, generating a small current, which is directly proportional to the BHBA concentration in the sample. It took approx. 10 s for each hand-held meter to present the analyzed BHBA concentrations on the display of the meters. The amount of blood required for analyses ranges between 0.8 µL (GLX, NOV) and 1.5 µL (FSP). The coccygeal blood samples were obtained with vacuum tubes coated with a clot activator for serum collection (Vacuette, 9ml, Greiner Bio-One GmbH, Kremsmünster, Austria). The samples were immediately tested with all 3 electronic devices in randomly order, too, by dipping the sensor of the strips onto the surface of the blood-filled tube. After clotting, the serum tubes were centrifuged (10 min, 18 °C, 2,200 x g) and serum was divided into 2 aliquots and stored at a temperature of -18 °C until further analyses at the laboratory of the Central Clinical Pathology Unit (CCPU), University of Veterinary Medicine, Vienna, Austria. The concentration of BHBA in serum was analyzed in the laboratory using a colorimetric enzymatic reaction (Ranbut D-3-hydroxybutyrate; Randox Laboratories Ltd., Antrim, UK) with an automated wet chemistry analyzer (Cobas 6000/501c; Roche Diagnostics International AG, Rotkreuz, Switzerland). This method is based on the oxidation of D-3-hydroxybutyrate to acetoacetate by the enzyme 3-hydroxybutyrate-dehydrogenase. The oxidation is coupled to an NAD indicator system. The induced color change is proportional to the BHBA concentration. The concentrations of BHBA determined in serum at the CCPU were defined as gold standard in our study.
To evaluate the intra-assay variability of the laboratory analyses, a subset of 20 aliquot blood samples, taken from one cow, were randomly placed between the samples obtained from the study animals. Intra- and inter-assay coefficients of variations (CV) were furthermore calculated for each type of hand-held devices. For this, 3 blood samples with different BHBA concentrations based on FSP measurements with low (0.3 mmol/L), medium (1.3 mmol/L) and high (2.1 mmol/L) BHBA concentrations were tested 10 times with one device (intra-assay) and additionally with 10 different devices of the same type (inter-assay).

2.2 Statistical analyses

SPSS Statistics for Windows (version 20.0; IBM Deutschland GmbH, Ehningen, Germany), MedCalc for Windows (version 12.4; MedCalc Software, Ostend Belgium) and BiAS for Windows (version 10.06; Epsilon-Verlag, Darmstadt, Germany) were used for statistical analyses. Level of significance was set at $P = 0.05$.

Spearman correlation coefficients ($\rho_s$) were calculated for the BHBA concentrations analyzed in capillary or coccygeal blood for each hand-held device and their corresponding BHBA concentration determined at the CCPU. A correlation coefficient describes a statistical relationship between data, but does not proof any agreement between the compared results and does not detect any constant or proportional difference between two methods (Bilic- Zulle 2011). Hence, the agreement between the results of each hand-held meter and the gold standard was evaluated using the method described by Bland and Altman (1986).

Furthermore, regression analyses as recommended by Passing and Bablok (1983) were conducted to compare the BHBA concentration, analyzed in capillary and coccygeal blood with all 3 electronic hand-held devices, with the laboratory results.

Receiver operating characteristics (ROC) analyses were performed to calculate best thresholds to detect SCK in capillary and coccygeal blood for each hand-held meter and sensitivities (Se) as well as specificities (Sp) were reported for all devices using these optimized thresholds. The area under the ROC curve (AUC) characterizes the quality of the thresholds based on the Se and Sp (Swets 1988). An AUC of 1 represents a perfect test; an AUC of 0.5 and below represents a worthless test.

The number of incisions needed using the 3 different lancets to obtain capillary blood were compared by applying a Fisher’s exact test.
3. Results

The mean BHBA concentration ± SD of the 20 samples that were analysed at the CCPU to calculate the intra-assay variability of measurements of the wet chemistry analyser was 1.19 ± 0.01 mmol/L, resulting in a coefficient of variation of 1.13%.

Considering the 240 animals, enrolled in this study, 34 (14.2%) were in first lactation, 97 (40.4%) in second lactation, and 109 (45.4%) animals in third or higher lactation. All animals were sampled within a period of 21 days ante partum up to 29 days post partum (median 7 days in milk; 25% Perzentile 3 and 75% Perzentile 13).

Thirtythree (13.8%) of the 240 samples analyzed at the CCPU exceeded a BHBA concentration in serum of 1.2 mmol/L and 18 (7.5%) the threshold of 1.4 mmol/L. Prevalence of SCK remained at similar levels with 14.8% and 8.1%, respectively, considering only animals within the first two weeks of lactation (n=149).

All lancets used in the study were eligible for capillary blood specimen collection, but differed in the total number of incisions needed \( (P = 0.047) \). Capillary blood could be obtained with first incision in 85\% \( (n = 68) \) using the SL, 95\% \( (n = 76) \) using the MT and in 96\% \( (n = 77) \) using the MC lancet. An additional second (and third) incision has to be performed for the SL in 9 (3) cases, using the MT in 4 (0) and the MC in 2 (1) cases.

In total, 240 capillary and coccygeal blood samples, each were analyzed with the hand-held devices. Type ‘E4’-error messages, indicating a “damaged sensor, incorrect application or quantity of the blood sample“, referring to the manufacturer’s manuals, were observed for the GLX device in 23 cases analyzing capillary blood, and in 10 cases for analyses of coccygeal blood. After consulting the manufacturer, a new batch of test strips was used for further analyses. Similar error messages were observed for the NOV device analysing capillary blood in 15 cases and for coccygeal blood in 4 cases. In case of an “E4”-failure, measurement was repeated.

The correlation \( (\rho_s) \) between the gold standard and the BHBA concentrations analyzed in coccygeal blood using the hand-held devices were 94.7\% for the FSP, 85.0\% for the NOV and 80.5\% for the GLX device \( (P < 0.01 \) for all devices). Testing capillary blood with the electronic devices yielded in \( \rho_s \) of 82.6\% for the FSP, 72.9\% for the NOV and 62.1\% for the GLX device \( (P < 0.01 \) for all devices). Further descriptive statistical parameters for the
BHBA concentrations analyzed in coccygeal and capillary blood using the hand-held devices as well as the laboratory results are presented in Table 1.

Table 1: Descriptive statistics of the β-hydroxybutyrate concentration analyzed in coccygeal and capillary blood using three different hand-held devices as well as in serum analyzed at the laboratory

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Serum^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 (mmol/L)</td>
<td>0.89</td>
<td>0.97</td>
<td>0.81</td>
</tr>
<tr>
<td>SD (mmol/L)</td>
<td>0.44</td>
<td>0.47</td>
<td>0.42</td>
</tr>
<tr>
<td>Median (mmol/L)</td>
<td>0.77</td>
<td>0.80</td>
<td>0.80</td>
</tr>
<tr>
<td>Interquartile range (mmol/L)</td>
<td>0.37</td>
<td>0.40</td>
<td>0.40</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 NOV: NovaVet (Nova Biomedical, Waltham, USA)

As shown in Figure 1, the median BHBA concentration of the samples was always smaller in capillary blood than in coccygeal blood, no matter which hand-held device was used.
Figure 1. Differences between the BHBA concentrations in serum analyzed at the laboratory (gold standard) and the concentrations measured with 3 different hand-held devices either in whole blood obtained from a tail vessel or in capillary blood. Within each box the black line represents the 50th percentile (median), the 25th and 75th percentiles are marked by the lower and upper hinges. Smallest and largest statistical values are represented by the end of the whiskers; outliers and extremes are depicted by o and *
Bland-Altman plots (Figure 2) between the gold standard and coccygeal blood demonstrated a positive bias for the FSP of $0.02 \pm 0.12$ mmol/L, and negative biases of $0.06 \pm 0.17$ for the NOV and $0.10 \pm 0.21$ for the GLX. Using capillary blood, biases of $+0.08 \pm 0.19$ for the FSP, $+0.01 \pm 0.43$ for the NOV and $-0.07 \pm 0.36$ for the GLX were detected.

**Figure 2.** Differences in BHBA concentrations measured with 3 different electronic handheld devices in tail vessel (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 $\pm 2$ SD.
For measurements in capillary and coccygeal blood, Passing-Bablok regression detected significant proportional and systematic differences for the FSP and GLX devices compared with the reference method (Table 2).

### Table 2: Differences between the BHBA 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>Bland-Altman</td>
</tr>
<tr>
<td></td>
<td>Slope ( b )</td>
<td>( CI_{95} )</td>
</tr>
<tr>
<td>FSP</td>
<td>0.84</td>
<td>0.79-0.90</td>
</tr>
<tr>
<td>GLX</td>
<td>0.80</td>
<td>0.71-0.90</td>
</tr>
<tr>
<td>NOV</td>
<td>0.90</td>
<td>0.80-1.00</td>
</tr>
</tbody>
</table>

1 FSP: FreeStyle Precision, (Abbott GmbH & Co. KG, Wiesbaden, Germany);
   GLX: GlucoMen LX Plus (A. Menarini GmbH, Vienna Austria);
   NOV: NovaVet (Nova Biomedical, Waltham, USA)

2 CI\(_{95}\): 95% confidence interval

The calculated confidence intervals for the intercepts \( a \) did not include 0 and the confidence intervals for the slopes \( b \) did not include 1. This indicated that significant systematic and proportional differences were observed between the results of both hand held devices and the gold standard and that the results cannot be regarded as interchangeable. Analyzing capillary blood with the NOV, the 95% confidence interval for the intercept \( a \) was 0.00 to 0.15 and the interval for the slope \( b \) 0.80 to 1.00; confidence intervals for coccygeal blood were 0.03 to 0.14 for \( a \) and 0.88 to 1.02 for \( b \). Hence, the results of the NOV analyzing capillary blood were comparable with the gold standard; analyzing coccygeal blood no proportional, but systematic differences were detected.
Receiver Operating Characteristics (ROC) analyses were performed based on BHBA thresholds of 1.2 mmol/L and 1.4 mmol/L analyzed at the CCPU to obtain best possible test characteristics for diagnosing SCK in capillary and coccygeal blood (Figure 3).

**Figure 3.** Receiver operating characteristics (ROC) analyses for three different hand-held devices for diagnosis of ketoses either in tail vessel or capillary blood, using serum BHBA concentrations of 1.2 mmol/L (left) or 1.4 mmol/L (right) as threshold for subclinical ketosis.

Optimized thresholds for the hand-held devices with corresponding test characteristics are presented in Table 3.
Table 3: Corresponding thresholds and performance of 3 hand-held meters to detect subclinical ketosis in capillary and coccygeal blood based beta-hydroxybutyrate concentrations in serum of 1.2 and 1.4 mmol/L, respectively

<table>
<thead>
<tr>
<th>Serum BHBA-threshold [mmol/L]</th>
<th>Device</th>
<th>Capillary blood</th>
<th>Coccygeal blood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Optimized device threshold [mmol/L]</td>
<td>AUC [% (CI95)]</td>
<td>Se [% (CI95)]</td>
</tr>
<tr>
<td>1.2</td>
<td>FSP 1.0</td>
<td>96 (92-98)</td>
<td>100 (90-100)</td>
</tr>
<tr>
<td></td>
<td>GLX 1.1</td>
<td>87 (82-91)</td>
<td>80 (63-92)</td>
</tr>
<tr>
<td></td>
<td>NOV 1.1</td>
<td>90 (86-94)</td>
<td>89 (73-97)</td>
</tr>
<tr>
<td>1.4</td>
<td>FSP 1.4</td>
<td>98 (95-99)</td>
<td>100 (83-100)</td>
</tr>
<tr>
<td></td>
<td>GLX 1.1</td>
<td>89 (84-93)</td>
<td>90 (68-99)</td>
</tr>
<tr>
<td></td>
<td>NOV 1.3</td>
<td>93 (89-96)</td>
<td>80 (56-94)</td>
</tr>
</tbody>
</table>

1 FSP: FreeStyle Precision, (Abbott GmbH & Co. KG, Wiesbaden, Germany); GLX: GlucoMen LX Plus (A. Menarini GmbH, Vienna Austria); NOV: NovaVet (Nova Biomedical, Waltham, USA)
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
Analysing capillary blood, thresholds for the 3 devices should be lowered between 0.1 to 0.2 mmol/L assuming a serum BHBA threshold of 1.2 mmol/L, and between 0 and 0.3 mmol/L applying a serum BHBA threshold of 1.4 mmol/L. Thresholds to detect SCK in coccygeal blood should be lowered by 0.2 mmol/L for the GLX and NOV assuming a serum BHBA concentration of 1.2 mmol/L, and by 0.1 mmol/L for both devices considering a serum BHBA concentration of 1.4 mmol/L as indicative for SCK. Using the FSP device to detect SCK in coccygeal blood, serum threshold could be used.

Except for the GLX used with capillary blood, overall accuracies of the hand-held devices were ‘excellent’ (AUC ≥ 0.9) for diagnosing SCK in capillary and coccygeal blood, compared with the gold standard of 1.2 and 1.4 mmol/L BHBA. Evaluated accuracies for the GLX used with capillary blood were classified as ‘good’ (AUC between 0.8 to < 0.9). Sensitivities ranged between 80% and 100% for capillary blood and between 85% and 100% for coccygeal blood. Corresponding Sp ranged between 76% and 95% with capillary blood and between 83% and 98% when coccygeal blood was used. Results for the AUC were in all cases highly significant and ranged between 87% (GLX) to 100% (FSP).

The calculated intra-assay and inter-assay CV for the 3 devices analyzing 3 different BHBA concentrations are presented in Table 4.
Table 4: Intra-assay and Inter-assay Coefficients of Variation for FreeStyle Precision (Abbott GmbH & Co. KG, Wiesbaden, Germany), GlucoMen LX Plus (A. Menarini GmbH, Vienna Austria) and NovaVet (Nova Biomedical, Waltham, USA) devices within low, medium, and high BHBA concentrations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Intra-assay</th>
<th></th>
<th>Inter-assay</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FSP³</td>
<td>GLX⁴</td>
<td>NOV⁵</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>Medium</td>
<td>High</td>
<td>Average</td>
</tr>
<tr>
<td>Mean [mmol/L]</td>
<td>0.36</td>
<td>1.51</td>
<td>2.31</td>
<td>1.39</td>
</tr>
<tr>
<td>SD [mmol/L]</td>
<td>0.05</td>
<td>0.08</td>
<td>0.10</td>
<td>0.08</td>
</tr>
<tr>
<td>CV² [%]</td>
<td>13.6</td>
<td>5.5</td>
<td>4.5</td>
<td>7.9</td>
</tr>
</tbody>
</table>

¹ Based on measurements using FreeStyle Precision (low = 0.3 mmol/L; medium = 1.3 mmol/L; high = 2.1 mmol/L).
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 NOV = NovaVet (Nova Biomedical, Waltham, USA)
Analyzing low (0.3 mmol/L) BHBA concentrations resulted in intra-assay- and inter-assay CVs of > 10% for all 3 devices. Average intra-assay CVs ranged between 7.9% (FSP) and 10.9% (NOV); average inter-assay CV between 7.2% (FSP) and 13.1% (NOV).
4. Discussion

To our knowledge, this is the first study, evaluating capillary blood as specimen for diagnosing SCK in dairy cows. Animals enrolled in this study were tested within the transition period, because of their greater risk for developing SCK compared to mid- and late-lactating cows. The prevalence of SCK, however, was lower than in most of the previous studies (Geishauser et al. 1998, McArt et al. 2012, Suthar et al. 2013). Even considering only cows within the first 2 weeks of lactation the prevalence of SCK, based on serum BHBA concentrations of 1.2 mmol/L and 1.4 mmol/L was low with 14.8% and 8.1%, respectively. The reason for these low prevalences might be the farmers’ awareness for SCK, resulting in continuous improvement and implementation of good herd health management procedures.

The differences in the total number of incisions needed with each lancet to obtain adequate amounts of blood for analyses are small, but statistically significant. The worst result was determined for the SL, which has the smallest blade width of all lancets tested. The results for the MT and MC, with equal blade widths, were similar. As a minimal amount of incisions is striven with regard to the animals’ welfare, it is recommended to use lancets with blade widths of at least 1.5 mm.

All 3 electronic devices used in this study were already evaluated in previous studies (Iwersen et al. 2013, Mahrt et al. 2014) with blood obtained from a tail vessel as test substrate. For the FSP, Iwersen et al. (2013) determined a $\rho_s = 94\%$ and a mean difference of $+0.04 \pm 0.15$ mmol/L, and for the GLX device a $\rho_s = 80.3\%$ and a mean difference of $-0.12 \pm 0.22$, respectively, compared with the laboratory results. For the NOV, Mahrt et al. (2014) reported a $\rho_s = 87.0\%$ and a mean difference of $-0.07 \pm 0.17$. Considering coccygeal blood, the determined correlation coefficients and biases of our study are similar to those already reported, indicating a good consistency in measurements of the electronic hand-held devices with varying environmental and farm conditions. The main intention of this study, however, was to test if capillary blood was suitable for diagnosing SCK using electronic hand-held devices.

Whereas the determined correlation coefficient for coccygeal blood were ‘very strong’ (Taylor 1990), the determined correlation coefficients for using capillary blood were approx. 12 percentage points lower for the FSP and the NOV, and 18 percentage points lower for the GLX, but still indicating a ‘strong’ (FSP and NOV) or ‘moderate’ (GLX) association. The differences in the correlation coefficients may not only be caused by the type of blood sample
but might also be influenced by the sampling procedure. According to the manufacturer’s manuals, it is important to avoid haemolysis of the blood sample because of its negative effect on the accuracy of the results. While obtaining capillary blood, it was sometimes necessary to squeeze the skin of the vulva in order to get an adequate amount of blood. According to the Clinical Laboratory Standards Institute (CLSI 2008), squeezing of the skin might increase the risk of haemolysis in capillary blood and could explain the poorer performance. Hence, further research is needed, to evaluate the association between the level of squeezing and the BHBA concentration obtained in capillary blood using hand-held devices.

With capillary blood as test substrate, the FSP and the NOV devices overestimated the laboratory results, whereas the GLX tended to underestimate the BHBA concentrations. From a clinical point of view, these mean variations were acceptable, especially with BHBA concentrations in serum of 1.2 mmol/L and 1.4 mmol/L, respectively, commonly used as decision limits for SCK. As presented in Figure 2, the discrepancy between the results of the devices and the laboratory increased with greater BHBA concentrations. This should be considered when interpreting the results.

The Passing-Bablok regression analyses revealed a systematic and a proportional difference for nearly all devices with both sample types (capillary and coccygeal blood).

Hence, ROC analyses were performed to calculate optimized thresholds for the detection of SCK in coccygeal and capillary blood using the electronic hand-held devices. Applying the optimized threshold, all devices were capable to detect SCK in capillary and coccygeal blood. The observed sensitivities and specificities were within of those studies conducted by Iwersen et al. (2013) and Mahrt et al. (2014), allowing a reliable monitoring of SCK in dairy cows. Additionally, the AUC reveals good performance for all devices to detect cows suffering from SCK, but results improved when a serum threshold of 1.4 mmol/L and coccygeal blood was used as sample material.

To evaluate the consistency of the test results, intra- and inter-assay coefficients of variations were calculated. The average intra-assay coefficients of variation for the FSP (7.9%) and the GLX device (8.3%) were acceptable due to repeatability whereas the overall coefficient of variation of 10.9% for the NOV hand-held meter was higher. In general, the intra- and inter-assay coefficients of variation improved with greater BHBA concentrations.
5. Summary

The objective of this study was to evaluate the suitability of capillary blood obtained by a minimal invasive lancet technique to detect subclinical ketosis (SCK) in dairy cows using 3 different electronic hand-held devices [FreeStyle Precision (FSP, Abbott), GlucoMen LX Plus (GLX, A. Menarini), NovaVet (NOV, Nova Biomedical)]. A total of 240 pairs of capillary and coccygeal blood samples were collected from dry and fresh lactating Holstein-Friesian cows. Capillary samples were obtained from the skin of the exterior vulva by using 1 of 3 different lancets; corresponding coccygeal blood samples by a blood collecting system. In all samples, the concentration of ß-hydroxybutyrate (BHBA) was immediately analyzed with all 3 hand-held devices used in randomly order. Additionally, serum harvested from the coccygeal blood samples was analyzed in a conventional laboratory and used as gold standard.

All lancets used in the study were eligible for capillary blood specimen collection, but differed in the total number of incisions needed. Spearman correlation coefficients between the BHBA concentrations in capillary blood and the gold standard were highly significant with 83% for the FSP, 73% for the NOV and 63% for the GLX. Using capillary blood, Bland-Altman plots demonstrated positive biases for the FSP of +0.08 ± 0.19 mmol/L and of +0.01 ± 0.43 for the NOV, as well as a negative bias of -0.07 ± 0.36 for the GLX. Receiver Operating Characteristics (ROC) analyses based on serum BHBA concentration of 1.2 mmol/L resulted in optimized thresholds for capillary blood of 1.1 mmol/L for the NOV and GLX devices, and in 1.0 mmol/L for the FSP. Based on these thresholds sensitivities (Se) and specificities (Sp) were of 89% and 84% for the NOV, 80% and 89% for the GLX and 100% and 76% for the FSP. Based on a serum BHBA concentration of 1.4 mmol/L ROC analyses resulted in optimized cut-offs of 1.4 mmol/L for the FSP (Se 100%, Sp 92%), 1.3 mmol/L for the NOV (Se 80%, Sp 95%) and of 1.1 mmol/L (Se 90%, Sp 85%) for the GLX.

Using the electronic hand-held devices to detect SCK in capillary blood, the observed correlation coefficients as well as the test characteristics were smaller compared to blood obtained from a coccygeal vessel, but still in a good range for an on-side test. Hence, introducing this procedure for ketosis monitoring in dairy cows is recommended, especially for countries in which farmers are not allowed to collect conventional blood samples.
6. Zusammenfassung


Alle in dieser Studie verwendeten Lanzetten waren für die Kapillarblutentnahme geeignet, unterschieden sich jedoch in der Anzahl der notwendigen Punktionen, die zur Gewinnung eines zur Messung ausreichenden Blutropfens nötig waren. Der Korrelationskoeffizient nach Spearman zwischen den BHB-Konzentrationen, welche mit den Schnelltestgeräten im Kapillarblut ermittelt wurden und den im Labor ermittelten BHB-Konzentrationen waren hoch signifikant mit 83% für das FSP, 73% für das NOV und 63% für das GLX. Sofern Kapillarblut zur Messung herangezogen wurde, zeigten die Bland-Altman Diagramme eine durchschnittliche positive Abweichung für das FSP von +0,08 ± 0,19 mmol/L und von +0,01 ± 0,43 für das NOV sowie eine negative Abweichung von -0,07 ± 0,36 für das GLX. Die auf einer Serum-BHB-Konzentration von 1,2 mmol/L basierenden Receiver Operating Characteristics (ROC) Analysen ergaben optimierte Geräte-Schwellenwerte zum Nachweis einer Ketose im Kapillarblut von 1,1 mmol/L für das NOV- und GLX-Gerät und von 1,0 mmol/L für das FSP. Die auf diesen Schwellenwerten basierenden Sensitivitäten (Se) und Spezifitäten (Sp) betrugen 89% und 84% für das NOV, 80% und 89% für das GLX sowie 100% und 76% für das FSP. Basierend auf einer BHB-Konzentration im Serum von 1,4 mmol/L ergaben die ROC Analysen optimierte Geräte-Schwellenwerte von 1,4 mmol/L für das FSP (Se 100%, Sp 92%), 1,3 mmol/L für das NOV (Se 80%, Sp 95%) und 1,1 mmol/L (Se 90%, Sp 85%) für das GLX.
List of abbreviations

SCK    Subclinical ketosis
FSP    FreeStyle Precision
GLX    GlucoMen LX Plus
NOV    NovaVet
SL     SafetyLancets special,
MT     Microtainer Contact-Activated Lancet
MC     MiniCollect Safety Lancets
CCPU   Central Clinical Pathology Unit
CV     coefficients of variations
ρs     Spearman correlation coefficients
ROC    Receiver operating characteristics
Se     sensitivities
Sp     specificities
AUC    area under the ROC curve
BHBA   β-hydroxybutyrate
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**Figure 1:** Differences between the BHBA concentrations in serum analyzed at the laboratory (gold standard) and the concentrations measured with 3 different hand-held devices either in whole blood obtained from a tail vessel or in capillary blood. Within each box the black line represents the 50th percentile (median), the 25th and 75th percentiles are marked by the lower and upper hinges. Smallest and largest statistical values are represented by the end of the whiskers; outliers and extremes are depicted by o and *

**Figure 2:** Differences in BHBA concentrations measured with 3 different electronic hand-held devices in tail vessel (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

**Figure 3:** Receiver operating characteristics (ROC) analyses for three different hand-held devices for diagnosis of ketoses either in tail vessel or capillary blood, using serum BHBA concentrations of 1.2 mmol/L (left) or 1.4 mmol/L (right) as threshold for subclinical ketosis