**TECHNICAL NOTE:** Validation of BHBCheck blood β-hydroxybutyrate meter as a diagnostic tool for hyperketonemia.

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<th>Journal:</th>
<th><em>Journal of Dairy Science</em></th>
</tr>
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<tr>
<td>Manuscript ID</td>
<td>JDS-17-13583.R1</td>
</tr>
<tr>
<td>Article Type:</td>
<td>Technical Notes</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>09-Oct-2017</td>
</tr>
</tbody>
</table>
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| Key Words: | ketosis, cowside diagnostic tool, transition cow |
Technical Note: Validation of BHBCheck blood β-hydroxybutyrate meter as a diagnostic tool for hyperketonemia in dairy cows

By Sailer et al.

Accurate cowside blood β-hydroxybutyrate detection meters are valuable tools for evaluating postpartum hyperketonemia. Optimal diagnostic tools must be sensitive and specific, as well as cost and labor effective to be implemented into routine testing protocols. We evaluated the use of the BHBCheck blood meter for use in ketosis detection compared to a laboratory assay and a previously validated meter. The BHBCheck meter performed similarly to previously validated meters and had adequate diagnostic accuracy for determining hyperketonemia in dairy cows.
TECHNICAL NOTE: Validation of the BHBCheck blood β-hydroxybutyrate meter as a diagnostic tool for hyperketonemia in dairy cows


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ABSTRACT

Accurate cowside blood β-hydroxybutyrate (BHB) detection meters are valuable tools for rapid diagnosis of hyperketonemia. The main objective of this study was to compare the blood BHB measured in whole blood by the BHBCheck meter (PortaCheck, Moorestown, NJ) to a previously validated meter, Precision Xtra meter (Abbott Laboratories, Abbott Park, Illinois), and a colorimetric laboratory assay. Samples (n=426) were collected from postpartum primiparous and multiparous Holstein cows (n=79 cows) enrolled in one of two experiments (Exp) with different sampling schedules (Exp 1: n=39 cows, 58 samples; Exp 2: n=40 cows, 368 samples). In both Exp, whole blood samples were collected from the coccygeal vessels after morning milking, prior to morning feeding. Blood samples were used immediately for BHB quantification via the BHBCheck meter and the Precision Xtra meter. Blood was also collected into evacuated tubes containing no additive (Exp 1) or Potassium Oxalate/Sodium Fluoride (Exp 2), which were centrifuged for serum or plasma separation and stored at -20°C for subsequent analysis. Laboratory quantification of BHB concentration was done by the BHB LiquiColor Assay (EKF Diagnostics - Stanbio; Boerne, TX; certified for serum and plasma). Data were analyzed by UNIVARIATE, CORR, FREQ, REG, and LOGISTIC procedures of SAS 9.4. Within this sample set, average parity was 3.3 lactations and DIM was 14 d. The proportion of samples classified as hyperketonemia (BHB ≥ 1.2 mmol/L) was 25, 28, and 31% as determined by the colorimetric assay, BHBCheck meter, and Precision Xtra meter, respectively. The correlation for BHBCheck meter BHB concentration compared to the colorimetric assay concentrations was r = 0.96 with a sensitivity of 91% and specificity of 93%. Correlation, sensitivity, and specificity of the
Precision Xtra meter concentrations were 0.97, 98%, and 92%, respectively. Bland Altman plots demonstrated minimal bias for both meters. Area under the ROC curve suggests adequate diagnostic accuracy of both meters. Overall, accuracy, sensitivity, and specificity of the BHBCheck meter was similar to the Precision Xtra meter and laboratory assay and is appropriate for use as a cowside diagnostic test for hyperketonemia in dairy cows. Keywords: Ketosis, cowside diagnostic tool, transition cow
During the postpartum period, dairy cows are at increased risk of developing metabolic disorders, largely due to negative energy balance (NEB) and subsequent mobilization of triglyceride (TG) from adipose tissue (Grummer, 1993; Drackley, 1999; Duffield, 2000). Fates of mobilized TG within the liver include complete oxidation to energy, incomplete oxidation to ketone bodies, or storage as TG (Grummer, 1993). Although some peripheral tissues can use ketone bodies as an energy source, production of ketone bodies beyond tissue usage results hyperketonemia (HYK). When untreated, HYK is associated with negative impacts on animal health, production, and profitability (Herdt, 2000; McArt et al., 2015). Hyperketonemia is defined as blood $\beta$-hydroxybutyrate ($\text{BHB}$) $\geq$ 1.2 mmol/L (Iwersen et al., 2009; McArt et al., 2012b; Gordon et al., 2013; 2017) and the average postpartum prevalence of HYK worldwide ranges from 15 to 22%, although it is highly variable by farm (Suthar et al., 2013; Chandler et al., 2015; Santschi et al., 2016). The average cost per case is $289 (McArt et al., 2015); however, early treatment can reduce the costs and negative outcomes (McArt et al., 2012a), making detection protocols that are comprised of labor- and cost-effective diagnostics essential to managing HYK. Previous validation of a handheld blood ketone meter, the Precision Xtra meter (Abbott Laboratories, Abbott Park, IL), provided a valuable quantitative tool for diagnosing HYK using a minimally invasive blood sample, that also had greater sensitivity and specificity than previously used milk and urine tests (Iwersen et al., 2009; Oetzel, 2004). Recently, several additional meters have been developed and some have been evaluated for potential use in bovine HYK diagnostics (Bach et al., 2016).
Validation of these diagnostic tests in a controlled setting, prior to field application, is essential. A relatively new meter that has not yet been validated for use in bovine HYK diagnostics is the BHBCheck meter (PortaCheck, Moorestown, NJ). This meter quantifies BHB in a 0.7 µL whole blood sample, which is less blood than other meters which require between 0.8 to 1.5 µL of blood, and quantification is completed in under 5 sec, compared to other meters that average 10 sec. We hypothesized that similar to other hand-held BHB diagnostic meters, the BHBCheck meter would provide adequate accuracy for HYK diagnostics in dairy cattle. The objectives of this study were 1) to determine the efficacy of the BHBCheck meter as a cowside BHB monitoring device by validating it against a colorimetric laboratory assay and 2) to compare the BHBCheck meter to an industry standard cowside meter (Precision Xtra).

We conducted two animal experiments that were approved by the University of Wisconsin-Madison College of Agricultural and Life Sciences Animal Care and Use Committee (protocol numbers A05467 and A01569). For both experiments (Exp), blood was collected from Holstein cows immediately following morning milking and prior to feeding. The samples from Exp 1 were collected from 39 primiparous and multiparous cows between 4 and 18 DIM during twice weekly sampling on a privately-owned dairy in south-central Wisconsin from May to June 2016. Experiment 1 was intended to provide a balanced dataset of an equal number of samples that were above and below the 1.2 mmol/L HYK threshold. This was accomplished by collecting whole blood from cows between 4 and 18 DIM into evacuated collection tubes without additives and immediately analyzing blood using the Precision Xtra test. When a cow with HYK was identified, she was included in the study along with the next cow that was negative for HYK. Whole
blood samples were also analyzed using the BHBCheck meter. Samples were collected over several farm visits and some cows contributed more than one sample to the dataset. Although the balanced design in Exp 1 resulted in a nonrepresentative sample set relative to the population prevalence, it avoided having a sample set with a minimal proportion of samples above the 1.2 mmol/L threshold. Whole blood tubes were then centrifuged (2,500 × g, 15°C, 15 minutes) and serum was aliquoted and stored into 1.5 mL microtubes at -20°C until subsequent analysis. Experiment 2 was conducted at the University of Wisconsin-Madison Dairy Cattle Center from May to October 2016. Blood samples were collected from 40 multiparous cows between 1 and 45 DIM into an evacuated tube without additives, and into an evacuated tube containing potassium oxalate and sodium fluoride. Whole blood from the tube without additives was immediately analyzed on the BHBCheck and Precision Xtra meter. The tube containing potassium oxalate and sodium fluoride was centrifuged (2,000 × g, 4°C, 15 minutes) and plasma was aliquoted into 1.5 mL microtubes, and stored at -20°C for subsequent analysis.

Serum samples from Exp 1 and plasma samples from Exp 2 were analyzed using the LiquiColor colorimetric assay (EKF Diagnostics - Stanbio, Boerne, TX; certified for serum and plasma collected with EDTA, heparin, or sodium fluoride) per the manufacturer’s protocol. Samples were quantified in duplicate and samples with intra-assay CV greater than 10% were reanalyzed. The inter-assay CV of the laboratory assay was 6.7%. Sample BHB concentration determined by the laboratory assay served as the “gold standard” BHB concentration for statistical analysis.
Data analysis was completed in SAS 9.4 (Cary, NC). Descriptive statistics were analyzed by PROC UNIVARIATE. Residuals were produced by PROC REG with colorimetric laboratory assay BHB as the dependent variable and BHBCheck or Precision Xtra meter quantified BHB as the independent variable. Pearson correlations between the BHB concentration determined by both meters and by the colorimetric assay were analyzed by PROC CORR. In order to account for correlation among residual errors due to the repeated measures on the experimental unit (cow) across time, different error structures (i.e. compound symmetry, first order auto-regressive, and spatial power) were tested using PROC MIXED. A log-likelihood ratio tests was performed between the repeated measure models and the null model and indicated accounting for repeated structure of the data did not significantly alter model fit. Therefore, analysis of the data continued without accounting for repeated measures. Sensitivity and specificity were calculated by PROC FREQ. Receiver operating characteristic (ROC) curves and associated area under the curve were determined by PROC LOGISTIC. Results for Exp 1 and 2 are provided both separately, and combined, in order to provide the diagnostic accuracy of the meters in two sample sets with different proportion of samples greater than or equal to 1.2 mmol/L.

Demographics of the samples from Exp 1, 2, and Overall are provided in Table 1. Samples were collected from a total of 79 Holstein cows with an average parity of 3.3 ± 0.1 and average DIM of 14.3 ± 0.6 across the two experiments (Table 1). The mean BHB was 1.3 ± 0.12, 1.0 ± 0.03, and 1.0 ± 0.03 mmol/L for samples from Exp 1, 2, and Overall, respectively. The range of BHB was from 0.3 to 5.4 mmol/L across all samples, as determined by colorimetric laboratory assay.
Proportion of samples with BHB ≥ 1.2 mmol/L, as determined by the colorimetric assay were 50% for Exp 1, 21% for Exp 2, and 25% for the Overall dataset. Use of either meter to calculate the proportion of samples with BHB ≥ 1.2 mmol/L resulted in slight overestimations in Exp 2 and Overall for the BHBCheck (50%, 25%, and 28% for Exp 1, Exp 2, and Overall) and in Exp 1, Exp 2, and Overall for the Precision Xtra (55%, 27%, and 31% for Exp 1, Exp 2, and Overall). This overestimation highlights the possibility of falsepositives when using a cowside BHB meter to diagnose HYK, and would result in treating a small number of cows that do not have blood BHB ≥ 1.2 mmol/L per the laboratory assay. Treating false positives does have a cost associated with it dependent on the disease and treatment; however, in the case of HYK, the cost of treatment is relatively low compared with the cost of an untreated HYK case (McArt et al., 2015).

Concentrations of BHB as determined by the BHBCheck or Precision Xtra meter were correlated \( (P<0.001) \) with concentrations determined by the colorimetric laboratory assay. Correlation coefficients were 0.98 for both meters compared with the laboratory assay in Exp 1. The correlation coefficients were 0.96 and 0.97, for the BHBCheck and Precision Xtra meter, respectively, in Exp 2 and the Overall dataset. Interestingly, the correlation between the two meters was 0.98 for Exp 1, 2, and Overall. Fit statistics for linear regression analysis of the BHBCheck and laboratory assay resulted in an \( r^2 \) and RMSE of 0.96 and 0.17 for Exp 1, 0.92 and 0.19 for Exp 2, and 0.93 and 0.19 Overall (Figure 1).

Linear regression analysis of the Precision Xtra meter compared with the laboratory assay resulted in an \( r^2 \) and RMSE of 0.97 and 0.17 for Exp 1, 0.94 and 0.17 for Exp 2, and 0.94 and 0.17 Overall (Figure 1). Bland Altman plots (Bland and Altman, 1986)
show test agreement (Figure 2) and demonstrate minimal bias for both meters (-0.05 and -0.09 for the BHBCheck and Precision Xtra meter, respectively, compared with the laboratory assay). Bias of -0.05 and -0.09 would not influence diagnostic ability of the test cowside and is consistent with minimal bias previously observed with cowside BHB diagnostic tests (Bach et al., 2016).

Although correlation and regression methods are valuable indicators of a diagnostic tool’s quantitative accuracy, it is equally important to evaluate the sensitivity and specificity of the meters at relevant diagnostic cutoff concentrations. Sensitivity and specificity of the Precision Xtra meter was 98 and 92% at 1.2 mmol/L for the Overall dataset in the current study (Table 2), which is consistent with previous validation of this meter (Iwersen et al., 2009; Voyvoda and Erdogan, 2010; Bach et al., 2016). Similarly, the BHBCheck meter had a sensitivity and specificity of 91 and 93% at the 1.2 mmol/L threshold. Sensitivity and specificity were greater in Exp 2 than Exp 1, likely due to the difference in sample size. Although both sensitivity and specificity are important indicators of a diagnostic tool, sensitivity is arguably more valuable for subclinical disorders with low-cost and low-risk treatments. Subclinical animals would fail to be diagnosed if missed by the diagnostic test, given the lack of obvious disease symptoms. In the case of HYK, the treatment cost of treatment is relatively inexpensive compared to the cost of an untreated case (McArt et al., 2015); thus, adequate sensitivity of a potential cowside diagnostic tool is important to consider (Bach et al., 2016).

Determination of the area under the ROC curve is an objective means of evaluating the overall validity of a diagnostic tool (Šimundić, 2009). Receiver operating characteristic curves represent the paired sensitivity and specificity for each cutoff, and
when plotted, the resulting curve allows for estimation of the discriminative power of a
test. Areas under the ROC between 0.9 and 1.0 represent “excellent” diagnostic accuracy
according to ROC diagnostic categories (Šimundić, 2009). In the current study, area
under the ROC for the BHBCheck meter was 0.98, 0.98, and 0.98 for Exp 1, Exp 2, and
Overall. The area under the ROC curve for the Precision Xtra meter was 0.98, 0.99, and
0.99 for Exp 1, Exp 2, and Overall. This evaluation indicates that both the Precision Xtra
and BHBCheck meters portray adequate diagnostic abilities.

Overall, performance of the Precision Xtra meter within the current study was
consistent with previous studies (Iwersen et al., 2009; Bach et al., 2016). The Precision
Xtra meter has been previously validated and has set the expectation for sensitivity and
specificity for a cowside BHB meter. While it is advantageous to have several diagnostic
options, diagnostic tools need to be evaluated to ensure test accuracy. Within the current
study, the BHBCheck meter performed similarly to the Precision Xtra meter in this study
and to other meters previously examined (Bach et al., 2016; Iwersen et al., 2009;
Voyvoda and Erdogan, 2010). Both meters were consistent with expectations of a
diagnostic tool and displayed adequate diagnostic accuracy. We conclude that the
BHBCheck meter is an accurate and valuable option for use in HYK detection protocols.

ACKNOWLEDGEMENTS

This research was partially funded by gifts from the Wisconsin Alumni Research
Foundation. BHBCheck meters and strips were provided by PortaCheck. The authors
would like to thank the University of Wisconsin-Madison Dairy Cattle Center staff as
well as the owner and herdsman of the privately-owned dairy for their cooperation during this research project.
REFERENCES


Table 1. Demographics of samples from experiment (Exp) 1 (collected between 4 and 18 DIM), Exp 2 (collected between 1 and 45 DIM), and Overall used to evaluate the cowside blood β-hydroxybutyrate meters\(^1\) compared with a colorimetric laboratory assay\(^2\).

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<th>Exp 1</th>
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<tbody>
<tr>
<td>Unique cows</td>
<td>39</td>
<td>40</td>
<td>79</td>
</tr>
<tr>
<td>Samples</td>
<td>58</td>
<td>368</td>
<td>426</td>
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<tr>
<td>Cow variables</td>
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<td></td>
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<tr>
<td>parity ± SE</td>
<td>2.7 ± 0.18</td>
<td>3.3 ± 0.08</td>
<td>3.3 ± 0.08</td>
</tr>
<tr>
<td>DIM ± SE</td>
<td>10.2 ± 0.56</td>
<td>15.1 ± 0.66</td>
<td>14.3 ± 0.58</td>
</tr>
<tr>
<td>BHB(^3), mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean ± SE</td>
<td>1.3 ± 0.12</td>
<td>1.0 ± 0.03</td>
<td>1.0 ± 0.03</td>
</tr>
<tr>
<td>median</td>
<td>1.2</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td>min</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>max</td>
<td>3.9</td>
<td>5.4</td>
<td>5.4</td>
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\(^1\)BHBCheck meter (PortaCheck, Moorestown, NJ) and Precision Xtra meter (Abbott Laboratories, Abbott Park, Illinois).

\(^2\)LiquiColor colorimetric assay (EKF Diagnostics - Stanbio, Boerne, TX).

\(^3\)As quantified by the colorimetric laboratory assay.
Table 2. Sensitivity and specificity of cowside blood β-hydroxybutyrate meters\(^1\) to correctly identify samples as greater than or equal to 1.2 mmol/L, based on a colorimetric laboratory assay\(^2\) within experiment (Exp) 1 (collected between 4 and 18 DIM), Exp 2 (collected between 1 and 45 DIM), and Overall.

<table>
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<tr>
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<th>Exp 2</th>
<th>Overall</th>
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<tbody>
<tr>
<td>Samples</td>
<td>58</td>
<td>368</td>
<td>426</td>
</tr>
<tr>
<td>Samples ≥ 1.2 mmol/L</td>
<td>29</td>
<td>77</td>
<td>106</td>
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\begin{tabular}{lccc}
BHBCheck & & & \\
Sens, %   | 89 [71,98] & 91 [84,96] & 91 [84,96] \\
Spec, %   | 84 [66,95] | 93 [89,95] & 93 [89,95] \\
Precision Xtra & & & \\
Sens, %   | 100 [87,100] & 97 [91,99] & 98 [93,99] \\
Spec, %   | 84 [66,95] | 92 [88,95] & 92 [88,94] \\
\end{tabular}

\(^1\)BHBCheck meter (PortaCheck, Moorestown, NJ) and Precision Xtra meter (Abbott Laboratories, Abbott Park, Illinois).

\(^2\)LiquiColor colorimetric assay (EKF Diagnostics - Stanbio, Boerne, TX).

\(^3\) [95% CI]
Sailer Figure 1. Linear regression of blood β-hydroxybutyrate (BHB) concentrations quantified by BHBCheck (PortaCheck, Moorestown, NJ; left panels) and Precision Xtra meter (Abbott Laboratories, Abbott Park, Illinois; right panels) compared with a colorimetric laboratory assay (LiquiColor colorimetric assay; EKF Diagnostics - Stanbio, Boerne, TX) within experiment (Exp) 1 (top panels; 58 samples, collected between 4 and 18 DIM), Exp 2 (368 samples, collected between 1 and 45 DIM), and Overall (bottom panels).

Sailer Figure 2. Differences of blood BHB concentrations determined in 426 samples (collected between 1 and 45 DIM across two studies) by a laboratory assay (LiquiColor colorimetric assay; EKF Diagnostics - Stanbio, Boerne, TX) and BHBCheck (PortaCheck, Moorestown, NJ; top panel) or Precision Xtra meter (Abbott Laboratories, Abbott Park, Illinois; middle panel) and between the two meters (bottom panel).
Sailer Figure 1.

\[ r = 0.98 \]

\[ r = 0.96 \]

\[ r = 0.96 \]

\[ r = 0.97 \]

\[ r = 0.97 \]
Sailer Figure 2.

![Graph](image)

Average BHB for Assay and BHBCheck, mmol/L

Average BHB for Assay and Precision Xtra, mmol/L

Average BHB for PrecisionXtra and BHBCheck, mmol/L