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ORIGINAL RESEARCH

# Characterization of the analytic performance of an electrochemical point-of-care meter for measuring β-hydroxybutyrate concentration in blood and plasma from periparturient dairy cattle

Ameer A. Megahed[1,2], Mark W. H. Hiew[1,3], Jonathan R. Townsend[1], Peter D. Constable[4]

[1]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Purdue University, West Lafayette, IN, USA; [2]Department of Animal Medicine (Internal Medicine), Faculty of Veterinary Medicine, Benha University, Benha, Egypt; [3]Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, Selangor, Malaysia; and [4]Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL, USA

**Key Words**
Bovine, hyperketonemia, ketosis

**Correspondence**
P.D. Constable, Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, 2001 S. Lincoln Ave, Urbana, IL 61802, USA
E-mail: constabl@illinois.edu

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**Background:** The Precision Xtra electrochemical meter is widely used to measure blood β-hydroxybutyrate concentration (BHBb) in dairy cattle. The meter uses an algorithm optimized for human blood that assumes the HCT in cattle is the same as in people, and that intra-erythrocyte β-hydroxybutyrate (BHBe) and plasma β-hydroxybutyrate (BHBp) concentration are equivalent.
**Objectives:** The first objective was to characterize the analytic performance of the meter for measuring BHBb and BHBp in dairy cattle. The second objective was to characterize the influence of HCT and sample temperature on BHBp concentration measured by the meter.
**Methods:** Blood and plasma samples were obtained from 106 periparturient Holstein cattle and 15 lactating Holstein cows with experimentally induced electrolyte and acid–base imbalances. Meter performance was evaluated using Deming regression and Bland–Altman plots. Multivariable linear regression was used to determine the effect of HCT and sample temperature on BHBb and BHBp concentration, respectively.
**Results:** The meter was linear up to BHB = 3.0 mmol/L as measured by the reference method, equivalent to meter values for BHBb > 4.5 mmol/L and BHBp > 5.2 mmol/L. An increase in HCT resulted in higher BHBb concentration. This result was partially explained by BHBe being much lower than BHBp. Changes in sample temperature caused a linear change in measured BHBp whenever BHBp > 3.0 mmol/L.
**Conclusions:** Meter accuracy was markedly dependent on the BHBe-to-BHBp ratio and consequently the HCT. Therefore, the algorithm used by the meter should be revised when applied to bovine blood for improved accuracy.

## Introduction

Ketosis is a common metabolic disorder of dairy cattle in early lactation that is characterized by increased concentrations of β-hydroxybutyrate (BHB), acetoacetate, and acetone in blood, plasma, serum, milk, and urine.[1,2] Measurement of plasma or serum BHB concentration is considered the gold standard test for diagnosing hyperketonemia in dairy cattle because it is the predominant and most stable of the 3 ketone bodies.[3–10] The most commonly used cutoffs or clinical thresholds for diagnosing hyperketonemia in dairy cattle are blood, plasma, or serum BHB concentration ≥ 1.0–1.4 mmol/L.[11–13]

The Precision Xtra meter was introduced in 2005 as an electrochemical point-of-care unit that uses specialized electrochemical test strips to monitor ketone and glucose concentrations in blood.[14,15]

The first layer of the test strip retains erythrocytes and facilitates passage of plasma by capillary action to an underlying layer that employs an enzymatic reaction, with the reaction products being detected by generation of an electrical current.[16–19] This analytic method assumes that the measured value for BHB is minimally affected by changes in HCT and sample temperature. Changes in HCT would influence the measured value for BHB if the intraerythrocyte β-hydroxybutyrate concentration (BHBe) differed from the plasma β-hydroxybutyrate concentration (BHBp). Interestingly, it has been known for more than 70 years that BHBe makes up approximately 1/3 to 1/2 the value of BHBp in human and sheep blood.[20–22] This strongly suggests that changes in HCT are likely to alter the measured BHB concentration in cattle blood. In addition, the results of a recent study indicated that the blood BHBb concentration measured by the meter of a blood sample at < 32°C was lower than that measured at 37°C.[23] The potential effect of sample temperature had not been explored in method comparison studies, where blood samples were usually obtained from the finger, ear, and foot pad, or by venipuncture, then immediately analyzed, and consequently assumed to be at approximately 37.0–38.5°C.

The Precision Xtra meter provides a practical point-of-care method to diagnose hyperketonemia in domestic animals because of the low cost of the unit and test strips, coupled with its ease of use and rapid test results. Preliminary reports of the performance of the meter in dairy cattle were published between 2006 and 2008.[24–26] More detailed method comparison studies in dairy cattle were subsequently published from 2009 to 2013.[23,27,28] None of these studies investigated the effect of HCT on the test performance or evaluated a wide range of BHB concentrations. The latter issue is of particular interest in that studies utilizing human, canine, and feline blood have demonstrated that the meter is nonlinear in blood when BHB > 3–4 mmol/L[29–33], and reaches a plateau at concentrations > 6 mmol/L in human blood[29,32] and > 3 mmol/L in canine blood.[31,34] Method comparison studies utilizing ovine and caprine blood have identified proportional and constant biases for the meter relative to reference methods for BHB.[35–37] Complete characterization of the analytic performance of the meter, including identifying the linear range, would improve the clinical utility of the meter in measuring the BHB in blood from dairy cattle.

The proprietary algorithm used by the Precision Xtra meter to calculate BHBp from the measured value in blood is optimized for human blood samples. We hypothesized that the algorithm used by the meter to calculate BHBp was inaccurate when analyzing bovine blood because the median HCT of bovine blood (34%) is lower than the median value for human blood (43%) and BHBe is < BHBp. The first objective of this study was therefore to characterize the analytic performance of the meter for the measurement of BHBb and BHBp in adult dairy cattle. The second objective was to characterize the effect of changes in HCT and temperature on the measured BHB concentration.

## Materials and Methods

The study design used a convenience sample of periparturient dairy cattle and in vitro studies that employed purposive sampling. Methods were approved by the Purdue Animal Care and Use Committee under protocol number 1201000598.

### Measurement of blood β-hydroxybutyrate concentration

Blood samples were obtained prospectively from 106 late periparturient Holstein cattle including 35 primiparous and 71 multiparous animals from the Purdue University Dairy Research and Education Center between May 2012 and March 2013. Late gestation cows were fed an acidogenic total mixed ration (TMR), and early lactation cows were fed a lactating cow TMR based on formulations recommended by the National Research Council (NRC, 2001).[38] Rations were fed once daily between 08:00 and 09:30 am and cattle were provided ad libitum access to water at all times.

Blood samples were obtained daily from the coccygeal vein or artery at approximately 09:00 am on days −4, −3, −2, −1, 0, 1, 2, 3, 7, 14, 21, and 28 relative to calving (day 0), as described elsewhere.[39] The study reported here was part of larger study investigating energy, calcium, and potassium homeostasis in the periparturient period, and the prediction of parturition and dystocia in Holstein-Friesian cattle. Further results have been published elsewhere.[39–42]

Immediately after blood collection, BHB was measured in a drop of nonheparinized blood from the tip of the vacutainer needle using an electronic hand-held meter (Precision Xtra; Blood Glucose and Ketone Monitoring System; Abbott Diabetes Care Inc., Alameda, CA, USA). Both coded ketone test strips that included a calibrator strip with each new box of test strips, and noncoded ketone test strips were used.

### Measurement of plasma β-hydroxybutyrate concentration at different temperatures, hematocrit, and plasma protein concentration

Heparinized blood samples from the 106 cows were transferred to a climate controlled laboratory area adjacent to the animal housing area. Hematocrit and total plasma protein (TPP) concentration were measured in triplicate as described.[39] Plasma was harvested and transferred into a polypropylene vial within one h of centrifugation and stored at −20°C for up to 9 months.

In order to provide a large range of BHBp for the exploration of the linearity of the assay, 60 plasma samples from 15 lactating Holstein cows with experimental fasting-induced hyperketonemia were also analyzed. The experimental details for these 15 cows have been described.[43]

The effect of sample temperature on the measured BHB concentration by the meter was investigated by purposively selecting 14 plasma samples from 10 periparturient cows with BHBp concentrations at 37°C ranging from 0.5 to 7.5 mmol/L in approximately 0.5 mmol/L increments. Plasma samples were placed in a water bath at 7, 12, 17, 22, 27, 32, 37, and 42°C for 30 min and then immediately analyzed in duplicate using the meter as previously described. This range of temperature was selected based on a working range of temperature (10–50°C) recommended by the manufacturer and because it included the ambient temperature range when the meter was used in the study reported here. At ambient temperatures < 4°C, the meter reports an error.

### Reference method for measuring plasma β-hydroxybutyrate concentration

A total of 164 plasma samples, comprising 104 plasma samples of the highest measured BHBb for each of the 106 cows and 4 plasma samples from each of the 15 cows with experimentally induced hyperketonemia were analyzed at the Veterinary Diagnostic Laboratory at the University of Illinois at Urbana-Champaign using commercially available controls and kits (Randox Laboratories Ltd., Charles Town, WV, USA). The reference method employed β-hydroxybutyrate dehydrogenase to react with BHB in the presence of NAD at 37°C to form acetoacetate and NADH. The change in NADH concentration was detected spectrophotometrically using a Beckman Coulter AU680 analyzer (Beckman Coulter, Inc., Brea, CA, USA) and was directly correlated with BHBp. The manufacturer reported that the intra-observer CV for human BHBp based on 20 consecutive analyses was 3.8% at 0.3 mmol/L and 3.8% at 1.2 mmol/L.

### Calculations

One kilogram of plasma contains approximately 930 g of plasma water and 70 g of TPP, based on a typical TPP concentration of 70 g/L. An important analytic issue is that BHB is distributed in the plasma water phase and not the protein phase of plasma. Direct reading point-of-care meters, such as the Precision Xtra meter, sense the amount of reactive BHB in the plasma water phase and therefore measure BHB content in terms of molality (mmol/kg of plasma water). Because it is preferable for clinical purposes to present BHB content as a molar concentration in plasma ($BHBp_{molar}$) in units of mmol/L of plasma, BHB molarity is calculated from the BHB molality in whole blood ($BHBb_{molal}$) using a standard equation that assumes fixed values for HCT and TPP concentration[44], such that:

$$BHBp_{molar} = BHBb_{molal} \times 0.93/\{(0.71 \times HCT/100) + (1 - HCT/100) \times 0.93\} \quad (1)$$

Equation (1) was developed for human blood and is based on an erythrocyte water content ($f_e$) of 0.71 times the intra-erythrocyte volume, and a plasma water content of 0.93 L/kg of plasma based on a typical TPP of 70 g/L.[39]

Equation (1) has a fundamental assumption that the BHB in intra-erythrocyte water expressed in terms of molality ($BHBe_{molal}$) is equivalent to the BHB in plasma water expressed in terms of molality ($BHBp_{molal}$), such that $BHBe_{molal} = BHBp_{molal}$. It is important to acknowledge that this assumption is probably not true because $BHBe_{molal}$ is likely to be $< BHBp_{molal}$ in cattle blood, as demonstrated for human and ovine blood.[20–22] In order to address the likely possibility that $BHBe_{molal} < BHBp_{molal}$, we recently developed the following novel general equation that accounts for the situation whereby $BHBe_{molal} \neq BHBp_{molal}$:[39]

$$BHBp_{molar} = BHBb_{molal} \times (1 - [PP]/1000)/\{(r \times f_e \times HCT/100) + (1 - HCT/100) \times (1 - [PP]/1000)\} \quad (2)$$

where $r$ is expressed as the ratio of erythrocyte-to-plasma molal concentrations (mg/kg of water), such that $r = BHBe_{molal}/BHBp_{molal}$. It should be noted that equation (2) reduces to equation (1) when $r = 1$

(indicating that $BHBe_{molal} = BHBp_{molal}$), $f_e = 0.71$, and TPP = 70 g/L.

As derived elsewhere[39], equation (2) can be rearranged to provide an expression in terms of *r*, such that:

$$r = \{(1 - [PP]/1000)/(f_e \times HCT/100)\} \times \{(BHBb_{meter}/BHBp_{meter}) - (1 - HCT/100)\} \quad (3)$$

where $BHBb_{meter}$ and $BHBp_{meter}$ represent the molar value reported by the meter (units of mmol/L) when analyzing blood or plasma, respectively. A median estimate for the value of *r* in cattle blood can therefore be calculated by using the experimentally determined value for $f_e$ in cattle blood (0.65)[45] and measuring the $BHBb_{meter}$ and HCT in a blood sample, centrifuging the sample, and harvesting the plasma, and then measuring the TPP and $BHBp_{meter}$.

### Sensitivity of meter reading to changes in hematocrit, plasma protein concentration, and *r*

The sensitivity of the dependent variable (percent error reading by the electrochemical meter) to the 4 independent factors HCT, TPP, *r*, and $f_e$ in equation (2) can be conveyed by a spider plot, which graphically depicts the relationship between the dependent variable and percentage change in one independent factor, while the remaining 3 independent factors are held constant at typical values.[46] The spider plot was created using equation (2) and typical values for the blood of healthy people (HCT = 43%, TPP = 70 g/L; $r = 1.0$; $f_e = 0.71$).[39]

### Statistical analysis

Data are expressed as median and range; $P < .05$ was assigned as being statistically significant. Linear regression was used to characterize the relationship between $BHBp_{meter}$ and temperature. Deming regression was used to evaluate the relationship between $BHBb_{meter}$ and the plasma BHB determined by the reference method ($BHBp_{reference}$), as well as between $BHBp_{meter}$ and $BHBp_{reference}$. The agreement between the meter and reference method was also examined using Bland–Altman difference plots.[47] Deming regression was also used to evaluate the relationship between $BHBb_{meter}$ and $BHBp_{meter}$ to confirm the implicit assumption in equation (3) that the relationship was linear with zero asymptote. Multivariable regression was used to investigate the effect of the independent variables $BHBp_{reference}$, HCT, and the interaction between $BHBp_{reference}$ and HCT on the dependent variable ($BHBb_{meter}$). The interaction term was dropped from the analysis if HCT and the interaction between $BHBp_{reference}$ and HCT were not significant. Statistical analyses were performed using SAS 9.3 (SAS Inc., Cary, NC, USA), Analyse-it$_{2.26}$ (Analyse-it Software Ltd., Leeds, UK), and an Excel spreadsheet (Microsoft Corporation, Redmond, WA, USA).

## Results

The intra-meter CVs from 15 consecutive analyses of blood samples from cows with a low (0.6 mmol/L), moderate (1.1 mmol/L), and high (2.5 mmol/L) BHBb were 4.4%, 2.9%, and 2.8%, respectively. The intra-meter CVs from 20 consecutive analyses of plasma samples from cows with a low (0.4 mmol/L), moderate (1.4 mmol/L), high (3.1 mmol/L), and very high (6.2 mmol/L) BHBp were 9.5%, 5.3%, 5.8%, and 5.7%, respectively.

### Effect of temperature

Median CV values for BHBp when the 14 samples were measured by the meter in duplicate at plasma temperatures of 7, 12, 17, 22, 27, 32, 37, and 42°C were 3.3%, 1.8%, 2.3%, 2.5%, 1.5%, 4.0%, 1.6%, and 1.2%, respectively. Variation in sample temperature from 7 to 42°C had no effect on the value for $BHBp_{meter}$ when the sample $BHBp_{meter}$ ranged from 0.4 to 3.0 mmol/L (Table 1). In contrast, sample temperature had a marked effect on $BHBp_{meter}$ when the

**Table 1.** Linear regression analysis results for 14 plasma samples from 10 periparturient Holstein cattle (2 plasma samples were obtained each from 2 cows) characterizing the effect of sample temperature (T) ranging from 7 to 42°C on the plasma β-hydroxybutyrate concentration ($BHBp_{meter}$) measured by an electrochemical point-of-care meter.

| $BHBp_{meter}$ at 37°C | *P* Value For Linear Regression Equation | $R^2$ | Linear Regression Equation ($y = a + bx$) |
|---|---|---|---|
| 0.4 | .13 | .33 | NS |
| 0.9 | 1.00 | .00 | NS |
| 1.6 | .80 | .01 | NS |
| 2.0 | .72 | .02 | NS |
| 2.6 | .14 | .32 | NS |
| 3.1 | < .001 | .89 | $BHBp_{meter} = 2.43 + 0.017 \times T$ |
| 3.9 | .006 | .71 | $BHBp_{meter} = 3.40 + 0.013 \times T$ |
| 4.5 | < .001 | .92 | $BHBp_{meter} = 3.66 + 0.023 \times T$ |
| 5.2 | .006 | .84 | $BHBp_{meter} = 4.16 + 0.029 \times T$ |
| 5.8 | .001 | .85 | $BHBp_{meter} = 3.91 + 0.049 \times T$ |
| 6.9 | .002 | .81 | $BHBp_{meter} = 5.69 + 0.038 \times T$ |

NS indicates not significant.

measured value was > 3.0 mmol/L at 37°C (Figure 1). Moreover, linear regression identified a 0.3–0.8% decrease in the slope value for every 1°C decrease in temperature > 3.0 mmol/L. Accordingly, because sample temperature was not measured when $BHBp_{meter}$ was determined in the dataset containing 106 cows, measured values for $BHBp_{meter}$ > 3.0 mmol in this dataset were excluded from method comparison studies.

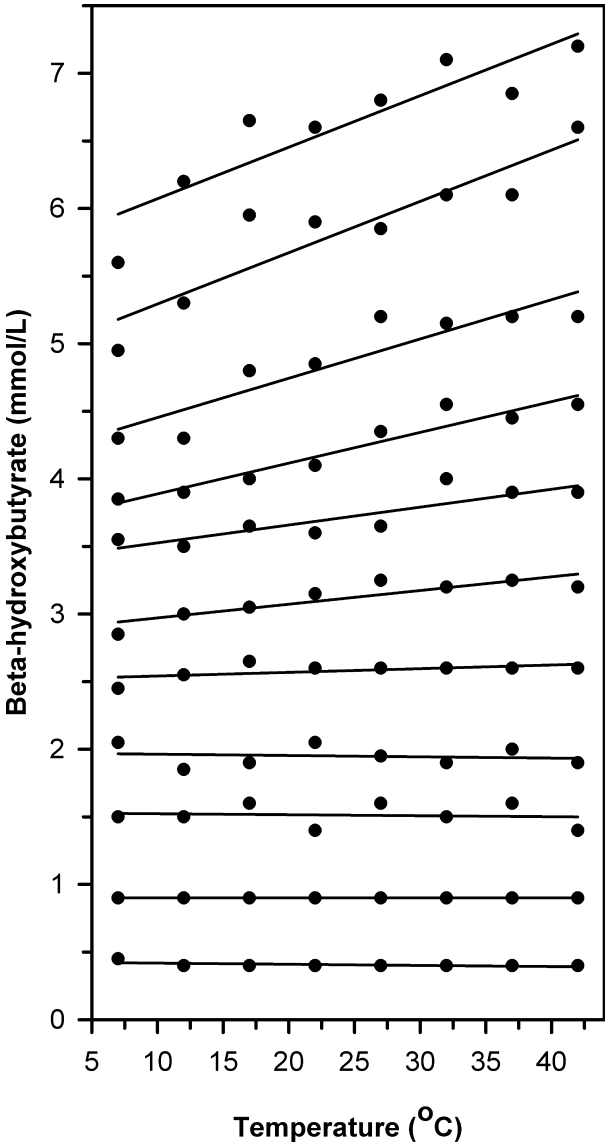


**Figure 1.** Scatterplots and linear regression line for 14 plasma samples from 10 periparturient Holstein cattle (2 plasma samples were obtained each from 2 cows) characterizing the effect of sample temperature ranging from 7 to 42°C on the plasma β-hydroxybutyrate concentration (BHBp) measured by an electrochemical point-of-care meter. Note that sample temperature has a significant linear effect on the measured value for BHBp whenever the measured BHBp at 37°C was > 3.0 mmol/L.

## Method comparison study

The $BHBp_{reference}$ in 164 plasma samples from 121 periparturient cattle ranged from 0.3 to 8.8 mmol/L (median, 0.9 mmol/L). Nonlinearity in the BHBb–$BHBp_{reference}$ relationship, as well as the BHBp–$BHBp_{reference}$ relationship, was identified using residual plots from linear regression (data not shown) when $BHBp_{reference}$ > 3.0 mmol/L (Figure 2).

Deming regression for BHBb against the reference method where $BHBp_{reference} \leq 3.0$ mmol/L (64 samples from 64 cows) indicated proportional bias (1.62; 95% CI, 1.40–1.83) and constant bias (−0.33 mmol/L; 95% CI, −0.51 to −0.14 mmol/L; Figure 3A). The meter was linearly related to $BHBp_{reference}$ but measured 0.8 mmol/L above to 0.3 mmol/L below the true value. Bland–Altman plots indicated that bias increased linearly as mean BHBb concentration increased (Figure 3B). Reorganization of the Deming regression equation produced the following equation for correcting the measured value:

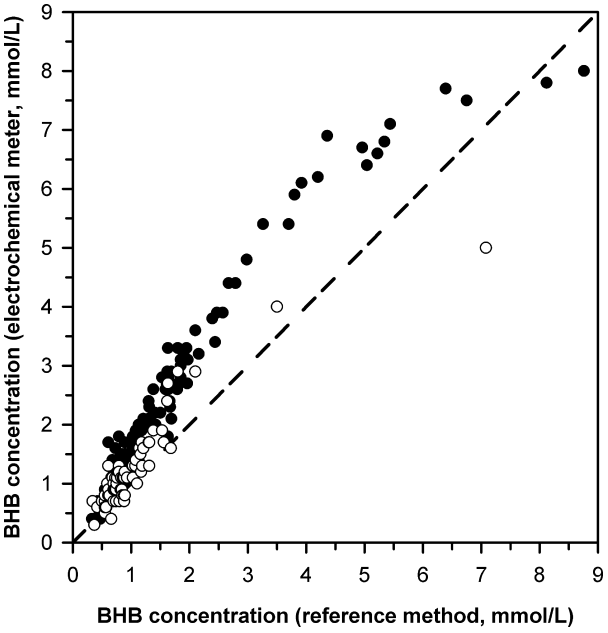


**Figure 2.** Scatterplot indicating the relationship between plasma β-hydroxybutyrate concentration (BHBp, filled circles, $n$ = 164) or blood β-hydroxybutyrate concentration (BHBb, open circles, $n$ = 74) measured by an electrochemical point-of-care meter vs the BHBp measured by the reference method for samples from periparturient Holstein cattle. Many points are superimposed because the meter reads to one decimal place. The dashed diagonal line is the line of identity.

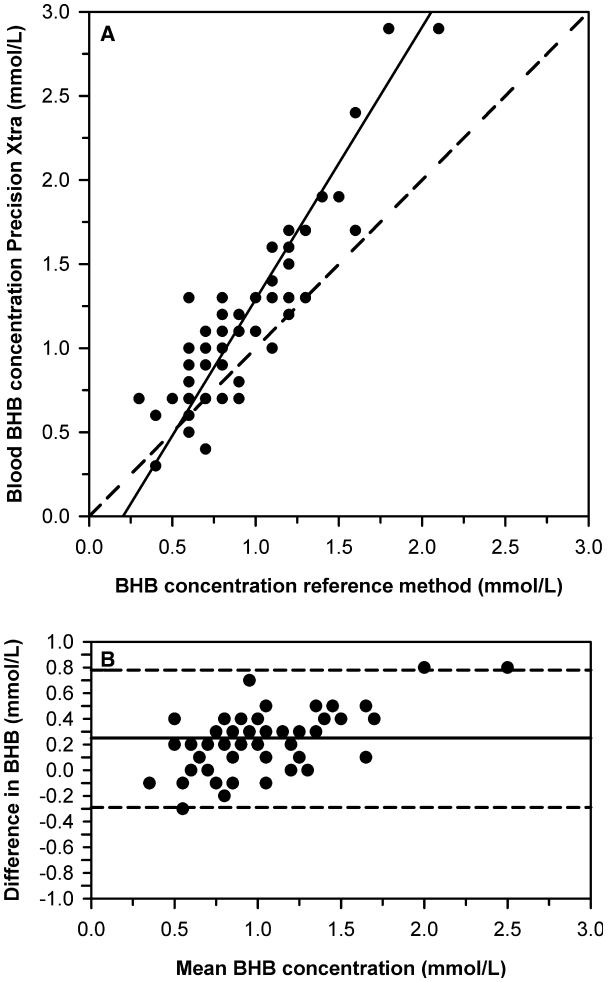


**Figure 3.** (**A**) Scatterplot indicating the relationship between blood β-hydroxybutyrate concentration (BHBb) measured by an electrochemical point-of-care meter vs the plasma β-hydroxybutyrate concentration ($BHBp_{reference}$) measured by the reference method for samples from 64 periparturient Holstein cattle. The dashed diagonal line is the line of identity, and the solid line is the line of best fit from Deming regression. Many points are superimposed because the meter reads to one decimal place. (**B**) Bland–Altman plot of the difference between BHBb measured by the electrochemical meter and the $BHBp_{reference}$ measured using the reference method against the mean BHB for both methods. The solid horizontal line is the mean bias (0.25 mmol/L) and the 2 horizontal dashed lines represent the 95% CI for agreement. The plot indicates that bias increased linearly as mean BHB increases.

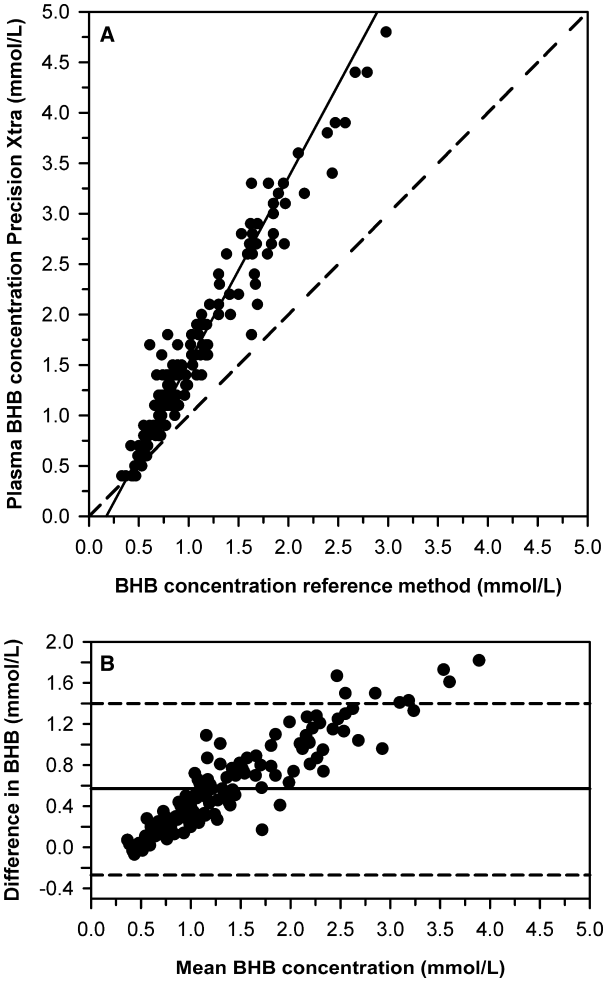


**Figure 4.** (**A**) Scatterplot indicating the relationship between plasma β-hydroxybutyrate concentration (BHBp) measured by an electrochemical point-of-care meter vs the $BHBp_{reference}$ measured by the reference method for 149 samples from 121 periparturient Holstein cattle. The dashed diagonal line is the line of identity, and the solid line is the line of best fit from Deming regression. Many points are superimposed because the meter reads to one decimal place. (**B**) Bland–Altman plot of the difference between BHBp measured by the electrochemical meter and the reference method against the mean BHB for both methods. The solid horizontal line is the mean bias (0.57 mmol/L) and the 2 horizontal dashed lines represent the 95% CI for agreement. The plot indicates that bias increased linearly as mean BHB increases.

$$BHB = 0.62 \times BHBb_{meter} + 0.20 \qquad (4)$$

Deming regression for BHBp against the reference method where $BHBp_{reference} \leq 3.0$ mmol/L (149 plasma samples from 121 cows) indicated proportional bias (1.84; 95% CI, 1.75–1.93) and constant bias (−0.32; 95% CI, −0.39 to −0.25 mmol/L; Figure 4A). The meter was linearly related to $BHBp_{reference}$ but measured 1.8 mmol/L above to 0.1 mmol/L below the true value. Bland–Altman plots indicated that bias increased linearly as mean BHBp increased (Figure 4B). Reorganization of the Deming regression equation produced the following equation for correcting the measured value:

$$BHB = 0.54 \times BHBp_{meter} + 0.17 \qquad (5)$$

Deming regression for $BHBb_{meter}$ against $BHBp_{meter}$ for 322 samples from 106 periparturient dairy cattle

where measured $BHBp_{meter} \leq 3.0$ mmol/L indicated a proportional bias of 1.36 (95% CI, 1.30–1.43) and a constant bias of −0.01 mmol/L (95% CI, −0.06 to 0.04 mmol/L; Figure 5A). This confirmed the implicit assumption in equation (3) that the relationship between $BHBb_{meter}$ and $BHBp_{meter}$ was linear with a zero intercept. Bland–Altman plots indicated that bias increased linearly as mean BHB concentration increased (Figure 5B).

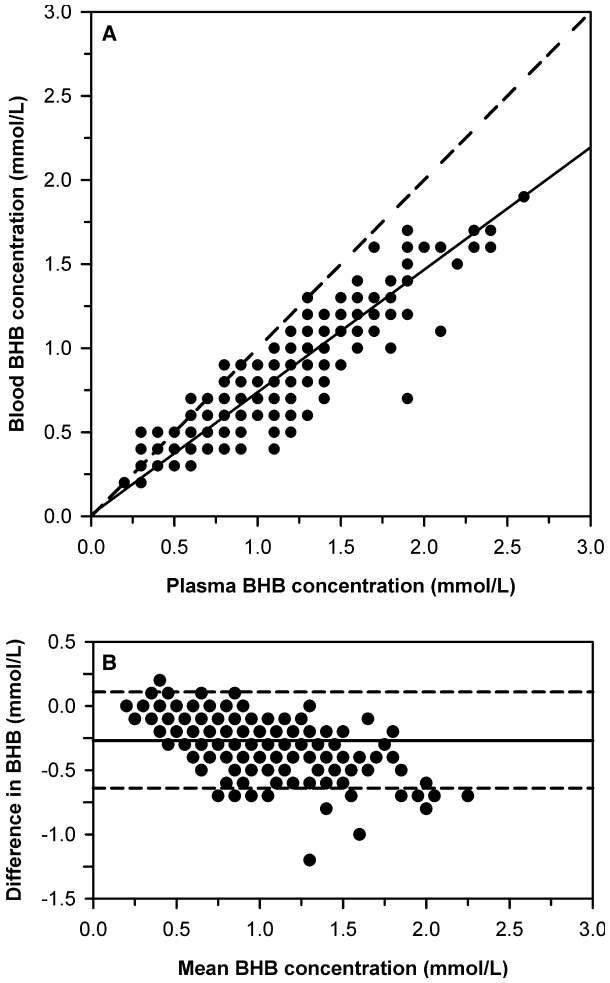


**Figure 5.** (**A**) Scatterplot indicating the relationship between blood β-hydroxybutyrate concentration (BHBb) and plasma β-hydroxybutyrate (BHBp) measured by an electrochemical point-of-care meter for 322 blood samples from 106 periparturient Holstein cattle. The thin diagonal line is the line of identity, and the solid line is the line of best fit from Deming regression. Many points are superimposed because the meter reads to one decimal place. (**B**) Bland–Altman plot of the difference between BHBb and BHBp concentrations measured by the meter against the mean BHB for both methods. The solid horizontal line is the mean bias (−0.27 mmol/L) and the 2 horizontal dashed lines represent the 95% CI for agreement. The plot indicates that bias decreased linearly as mean BHB concentration increased.

## Calculation of r and the ratio of intra-erythrocyte BHB to plasma BHB concentration

The median value for *r* was .37 for 322 paired blood and plasma samples from 106 periparturient dairy cattle with $BHBp_{meter} \leq 3.0$ mmol/L (the cutoff above which measured BHB was influenced by the plasma temperature). Hematocrit ranged from 23% to 41% (median, 33%) and TPP ranged from 30 to 84 g/L (median, 62 g/L). Substituting the calculated median value for *r*, the measured median value for TPP, and the experimentally determined value for $f_e$ (0.65) into equation (2), and subsequent algebraic rearrangement, produced the following equation that should be compared to equation (1):

$$BHBp_{molar} = BHBb_{molal} \times 0.94/\{(0.24 \times HCT/100) + (1 - HCT/100) \times 0.94\} \quad (6)$$

## Effect of hematocrit

Multivariable regression of $BHBb_{meter}$ against $BHBp_{reference}$, HCT, and the interaction between $BHBp_{reference}$ and HCT indicated that the interaction between $BHBp_{reference}$ and HCT was a significant predictor of $BHBb_{meter}$ (Table 2).

## Sensitivity of meter reading to changes in hematocrit, plasma protein concentration, and *r*

Sensitivity analysis using a spider plot (Figure 6) indicated that the percent error in the measured value for $BHBb_{meter}$ was most dependent on the value for *r*, moderately dependent on the HCT, and only minimally dependent on the TPP.

## Calculation of sensitivity and specificity at selected cutoffs

Hyperketonemia in lactating dairy cattle has most commonly been defined as blood, plasma, or serum

**Table 2.** Results of multivariable regression analysis characterizing the effect of plasma β-hydroxybutyrate concentration ($BHBp_{reference}$) determined by the reference method, HCT, and the interaction between $BHBp_{reference}$ and HCT on the value for blood BHB concentration measured by an electrochemical point-of-care meter in 64 periparturient Holstein cattle.

| Factor | Regression Coefficient | Standard Error | Probability |
|---|---|---|---|
| Intercept | −2.8 | 1.1 | .01 |
| $BHBp_{reference}$ | 4.9 | 1.4 | < .001 |
| HCT | 8.5 | 3.4 | .02 |
| $BHBp_{reference}$ × HCT | −11.2 | 4.2 | .01 |

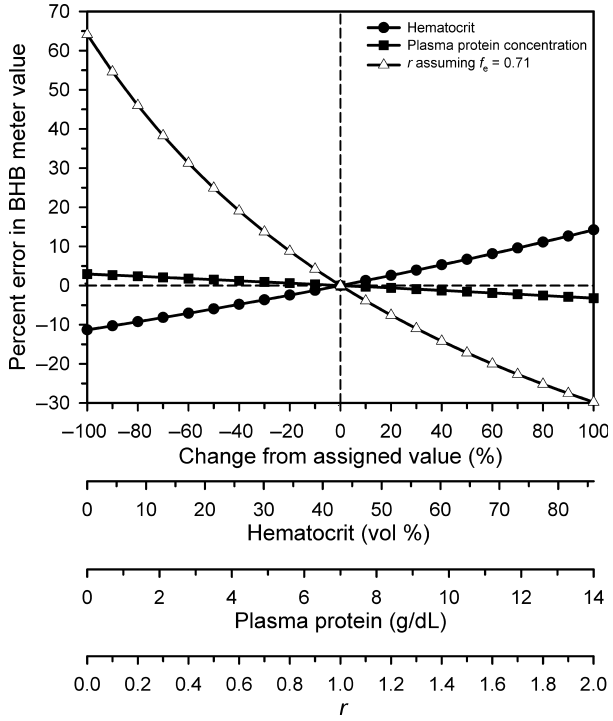


**Figure 6.** Spider plot revealing the dependence of percent error reading by an electrochemical point-of-care meter on the effect of changes in 4 independent variables (HCT; plasma protein concentration; *r*, the ratio of erythrocyte-to-plasma molal concentrations; and $f_e$, the erythrocyte water content) on the measured value for blood β-hydroxybutyrate concentration (BHBb). The spider plot was obtained by systematically varying one independent variable while holding the other 3 independent variables at their assumed values for human blood. Reference values for the 4 independent variables were as follows: HCT = 43% (filled circles), plasma protein concentration = 70 g/L (filled squares); $r$ = 1.0 (open triangles); $f_e$ = 0.71 (open triangles, same scale as $r$ on the $x$ axis). The dashed vertical and horizontal lines indicate that the percent error = 0% when HCT, plasma protein concentration, $r$, and $f_e$ are at their reference values.

BHB concentration ≥ 1.0, 1.2, or 1.4 mmol/L. Point estimates for the sensitivity and specificity of meter measured BHBb from 70 samples were: Se = 1.00 and Sp = 0.64 for BHB ≥ 1.0 mmol/L; Se = 1.00 and Sp = 0.75 for BHB ≥ 1.2 mmol/L; and Se = 1.00 and Sp = 0.86 for BHB ≥ 1.4 mmol/L. For comparison, point estimates for the sensitivity and specificity of BHB calculated using equation (4) from Precision Xtra meter measurements of 70 blood samples were: Se = 0.87 and Sp = 0.99 for BHB ≥ 1.0 mmol/L; Se = 0.64 and Sp = 1.00 for BHB ≥ 1.2 mmol/L; and Se = 0.50 and Sp = 1.00 for BHB ≥ 1.4 mmol/L. Similarly, point estimates for the sensitivity and specificity of BHBp calculated using equation (5) from meter measurements of 121 plasma samples were: Se = 0.92 and Sp = 0.96 for BHBp ≥ 1.0 mmol/L; Se = 0.79 and Sp = 0.99 for BHBp ≥ 1.2 mmol/L; and Se = 0.60 and Sp = 0.98 for BHBp ≥ 1.4 mmol/L.

## Discussion

The major and novel findings in the study reported here are that the BHB measured by an electrochemical point-of-care meter is dependent on the BHBe-to-BHBp ratio and therefore the HCT, and to a lesser extent is dependent on the TPP concentration. These dependencies are expressed graphically by the use of a spider plot, which emphasizes the dominant role that the intra-erythrocyte-to-plasma ratio of the analyte plays in determining the measured value for BHB concentration, and expressed algebraically in equation (2). An additional new finding in the study reported here was that the BHB meter was linear only up to 3 mmol/L when used to analyze bovine blood. Finally, we were able to confirm and extend preliminary findings[23] that sample temperature influenced the value for BHBp measured by the meter.

This study used a much wider range of BHBp values (0.3–8.8 mmol/L) than that used previously in method validation studies in cattle[23,27,28] and spanned the assay range of the meter (0.0–8.0 mmol/L) stated in the meter user's manual; however, the sample size used in this study reported here was lower than that used in the other studies.[23,28] A wide analytic range is vitally important in method comparison studies as it assists in detecting the presence of nonlinearity, as demonstrated in this study for the first time for BHB in bovine blood. Our finding of nonlinearity in bovine blood when true BHBp > 3.0 mmol/L, equivalent to $BHBb_{meter}$ > 4.5 mmol/L, and $BHBp_{meter}$ > 5.2 mmol/L was similar to findings in studies utilizing human, canine, and feline blood.[29–34] Nonlinearity at high BHB concentrations therefore appears to be an inherent property of the meter's analytic method. The clinical relevance of this finding is that values for $BHBb_{meter}$ > 4.5 mmol/L provide an underestimate of the true value. Based on our experience, very high values for BHBb or BHBp are predictive of mortality in dairy cattle with fat mobilization syndrome (severe hepatic lipidosis), and most clinicians are unaware that the BHBb or BHBp can reach 10 mmol/L in severely ketotic cows. The results of this study will hopefully alert dairy veterinarians to the potential clinical value in sequentially measuring BHB in critically ill dairy cows with hepatic lipidosis.

Our finding that the calculated value for $BHBe_{molal}$ was much lower than $BHBp_{molal}$ in cattle

blood (based on $r < 1$) was consistent with previous findings in human and ovine blood.[20,21,48–50] As a result, HCT will have an effect on the accuracy of the Precision Xtra meter for monitoring BHB in dairy cattle, as demonstrated by the spider plot and results of multivariable regression analysis in the study reported here, whereby a decrease in HCT falsely decreased the measured BHBb concentration, and an increase in HCT falsely increased the measured BHBb concentration. The covered HCT range is reported to be 30–60% in the meter user's manual. The HCT range of cattle blood in this study was 23–41% (median, 33%), and consequently some cattle had HCT values below the recommended range for the meter.

We also found that the sample temperature altered the measured BHBp concentration by the electrochemical meter when the measured BHBp > 3.0 mmol/L. This finding indicates that when used in research studies to measure $BHBp_{meter}$, the temperature of the plasma will not impact the accuracy of equation (5) for correcting the measured concentration as long as $BHBp_{meter} < 3.0$ mmol/L. The effect of temperature on the accuracy of the meter for monitoring BHB in dairy cattle has only been investigated in one other study.[23] That study was not designed to determine whether the effect of sample temperature depended on the measured value for $BHBb_{meter}$ because the investigators expressed their data as percent change from baseline. Enzymatic reactions can be influenced by the temperature and number of moles of reactants in the analyzed sample, unless reagents are in marked excess of reaction requirements.[51] In order to minimize the effect of temperature on enzyme activity, manufacturers of electrochemical strips usually provide a marked excess of enzyme so that the temperature dependency of the reaction speed is eliminated.[51] As a result of this strategy, electrochemical BHB strips are primarily limited by the rate of diffusion of plasma to the electrodes which are layered beneath the chemistry layer containing β-hydroxybutyrate dehydrogenase and NAD. The rate of plasma diffusion is sensitive to temperature, decreasing by approximately 2% for every 1°C decrease in temperature.[51]

A potential limitation of this study was the storage time of plasma samples at −20°C for up to 9 months until BHBp was measured by the reference method. However, BHBp is considered to be very stable at −20°C, with stability being demonstrated for at least 40 days[52], 2 months[3], and 6 months.[53] A second potential limitation of the study is that we investigated the effect of sample temperature on the accuracy of the meter using purposive sampling. This sampling approach was based on the operational construct that low sample temperature would result in a lower measured value for $BHBp_{meter}$, particularly in markedly hyperketonemic samples. Purposive sampling is susceptible to bias because samples are not randomly selected from the population and are therefore not always representative of the population. However, purposive sampling is appropriate when conducting initial exploratory investigations related to proof of concept, as in this study.[54] Based on our preliminary findings, more detailed studies characterizing the effect of temperature on the measured value for $BHBp_{meter}$ using stratified random sampling and other appropriate study designs appear indicated.

When the meter was applied to whole blood, point estimates for Se (1.00) and Sp (0.86) were highest at a cutoff of ≥ 1.4 mmol/L. Point estimates of Se (1.00) and Sp (1.00) for the meter had been previously identified at a cutoff of ≥ 1.4 mmol/L.[27] Another study[28] identified the highest values for Se (0.90) and Sp (0.98) for the meter at a cutoff of ≥ 1.4 mmol/L. Collectively, the results of the 3 method validation studies in cattle indicate excellent test performance of the meter at $BHBb_{meter} \geq 1.4$ mmol/L, and for the clinical identification of hyperketonemia, it is therefore recommended that the meter be used at $BHBb_{meter} \geq 1.4$ mmol/L. Interestingly, a $BHBb_{meter}$ value of ≥ 1.4 mmol/L is equivalent to a true plasma value of ≥ 1.07 mmol/L. It is important to note that test specificity was improved at cutoffs of ≥ 1.0, 1.2, and 1.4 mmol/L when equations (4) or (5) were used to correct the meter's measured concentration in blood or plasma, with a concomitant decrease in test sensitivity.

In conclusion, the Precision Xtra meter uses an algorithm optimized for analyzing human blood and consequently demonstrates some inaccuracies when used to analyze cattle blood. First, the meter is nonlinear at true BHBp > 3.0 mmol/L, equivalent to $BHBb_{meter} > 4.5$ mmol/L, and $BHBp_{meter} > 5.2$ mmol/L. Values reported by the meter that exceed these cutoffs should be regarded as underestimates of the true value. Hematocrit influences the accuracy of the meter, with a HCT < 43% (very common in cattle) resulting in underestimation of the true value. The accuracy of the meter can be improved by using equation (4) when measuring blood in the field or by using equation (5) when measuring plasma samples in research studies. Sample temperature of plasma also impacts meter accuracy, with underestimation of the true BHB occurring whenever the sample temperature of plasma is < 37°C and $BHBp_{meter} > 3.0$ mmol/L.

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