PAPER

# Point-of-care β-hydroxybutyrate measurement for the diagnosis of feline diabetic ketoacidaemia

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**Objectives:** To evaluate accuracy and precision of a hand-held ketone meter measuring β-hydroxybutyrate and to determine its diagnostic performance to rule out ketoacidaemia in diabetic cats.

**Methods:** The ketone meter was validated by calculating within-day precision at different β-hydroxybutyrate concentrations and by comparison with a laboratory method.
To determine its diagnostic performance to diagnose ketoacidaemia, 217 sets of data (venous blood gas analysis and β-hydroxybutyrate measurements) were retrospectively analysed. Sensitivities and specificities were calculated with the help of receiver-operating characteristic curves.

**Results:** The ketone meter reliably detected β-hydroxybutyrate at concentrations >0·1 mmol/L and reproducibility was acceptable. Measurements highly correlated with laboratory results (r=0·97; P<0·001), but a significant negative bias was found at high concentrations. A β-hydroxybutyrate concentration of >2·55 mmol/L had a sensitivity of 94% and a specificity of 68% for diagnosing ketoacidaemia. Many cats with high β-hydroxybutyrate concentrations and normal blood pH had an elevated chloride gap suggestive of superimposed hypochloraemic metabolic alkalosis.

**Clinical Significance:** The commercially available point-of-care ketone meter Precision Xtra is a valid tool to measure β-hydroxybutyrate in diabetic cats. Concentration <2·55 mmol/L enable ketoacidaemia to be excluded and should lead to redirection of differential diagnoses.

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## INTRODUCTION

Ketonaemia leading to life threatening ketoacidaemia (DKA) is a common emergency in newly diagnosed and treated diabetic cats. As clinical signs are non-specific (Crenshaw and Peterson 1996), the diagnosis is currently based on the biochemical triad of hyperglycaemia, a positive urine or plasma dipstick result measuring acetoacetate and a high anion gap (AG) metabolic acidosis ($AG=[Na^+ + K^+]-[Cl^- + HCO_3^-]$). The measurement of ketones is crucial as high AG acidosis is also seen in cats with hyperlactataemia or deteriorating chronic renal failure (Elliott and others 2003b). The current gold standard is the semi-quantitative measurement of urinary acetoacetate by nitroprusside reaction. The main problems with this approach are that urine is not always available and false negative results are possible (Stojanovic and Ihle 2011). To bypass these drawbacks plasma acetoacetate measurements have been advocated (Brady and others 2003, Zeugswetter and Pagitz 2009). In human medicine, quantitative β-hydroxybutyrate (β-OHB) determinations have widely replaced semi-quantitative acetoacetate measurements. The rationale is that the dipstick tests are unreliable and β-OHB becomes the dominant ketone during diabetic ketoacidosis (Laffel 1999). Inexpensive, easy to use small volume point-of-care ketone sensors for rapid near patient testing have become commercially available. Cut-off values to identify impending or established ketoacidosis have been established in humans (Wallace and others

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2001, Charles and others 2007, Voulgari and Tentolouris 2010) and dogs (Di Tommaso and others 2009), but not in cats. The objective of this study was to validate the point-of-care blood ketone meter Precision Xtra and to find a species specific cut-off value for this ketone meter above which buffering capacities are overwhelmed and metabolic decompensation occurs.

## MATERIALS AND METHODS

### Analytic evaluation of the ketone meter

Whole blood β-OHB was measured with the commercially available hand held ketone meter Precision Xtra (Abbott/MediSense) recently introduced to the veterinary market (Hoenig and others 2008). This ketone meter produces an electrical current proportional to blood β-OHB concentrations and gives a quantitative measurement of β-OHB in a range of 0·1 to 6 mmol/L. β-OHB concentrations >6 mmol/L are displayed as "HIGH". Ten microlitres of a whole blood sample are applied on the sensor and the concentrations are displayed after 30 seconds. For validation of the ketone sensor β-OHB values of 43 cats were measured three times in series and the mean was compared with a laboratory reference method (Ranbut; Randox Laboratories Limited; Zeugswetter and others 2010). Two representative samples with low, medium and high β-OHB concentrations were measured 10 times in sequence within 15 minutes to assess within- day precision.

### Diagnostic performance

Acid-base values, plasma electrolyte concentrations and β-OHB concentrations obtained on 217 occasions from 138 cats with newly diagnosed or insulin-treated diabetes mellitus (DM), presented between 2005 and 2011 at the local clinic, were retrospectively analysed. Serial measurements from the same admission or data from cats pretreated with alkalinising products were excluded. The study population consisted of 92 (67%) castrated male and 46 (33%) female cats with a median age of 10 years (range 1 to 19 years). In accordance with the local breed distribution domestic shorthair (n=127; 92%) and Persian (n=5, 3·6%) cats were overrepresented. The diagnosis of DM was based on patient history, clinical signs and repeated blood glucose and in most cases fructosamine measurements. Hyperglycaemia and hyperfructosaminaemia were defined as a glucose >8·3 mmol/L (150 mg/dL) and fructosamine >340 µmol/L (Hitachi 911 chemistry analyser until 2009, than Cobas c501, Hitachi), respectively. Blood gas and electrolyte analysis was performed with the Synthesis 25 (Instrumentation Laboratory) using ion-specific electrodes.

Sets of data were included if blood gas analysis and β-OHB measurements had been performed concurrently. The study was possible since β-OHB, pH and bicarbonate ($HCO_3^-$) measurements as well as the calculation of the AG are an integral part of the initial workup and monitoring of diabetic cats with possible ketoacidosis at the local clinic. On the basis of the acid-base status, cats were assigned to one of two groups: group 1=diabetic ketoacidaemia (DKA, ketonaemia associated with metabolic, high AG acidaemia); group 2=other. Metabolic, high AG acidaemia was defined as a pH≤7·27, $HCO_3^-$≤14 and an AG>20·6 (Elliott and others 2003a). Ketonaemia was defined as β-OHB >0·2 mmol/L, the highest value measured in 25 healthy clinic-owned cats. All cats in group 1 had clinical signs compatible with DKA and responded to intensive fluid and insulin therapy. Cats with high β-OHB concentrations but a normal pH and/or normal $HCO_3^-$ and/or normal AG were further evaluated. Corrected chloride was calculated with the formula: $[Cl^-]$ corrected=$[Cl^-]$ patient×156/$[Na^+]$ patient. Chloride gap was calculated with the formula: $[Cl^-]$ gap=120–$[Cl^-]$ patient×156/$[Na^+]$ patient. A $[Cl^-]$ gap >4 mmol/L reflects hypochloraemic metabolic alkalosis (DeMorais and Leisewitz 2006).

## STATISTICS

Data analysis was performed with the laboratory software package sigma-plot version 11 (Systat Software GMBH) and SPSS version 14 (SPSS Inc). All data were assessed for normality by the Kolmogorov-Smirnov test. Linear regressions were calculated using Spearman's correlation. Results obtained from the ketone meter and the reference laboratory method were compared with the Wilcoxon's paired *t* test. Readings displayed as "HIGH" were taken as 6 mmol/L. The overall performance of β-OHB measurements to identify cats with DKA was assessed by creating a receiver-operating characteristic (ROC) curve and calculating the area under the curve (AUC). An area under the ROC-curve of 1·0 was considered a perfect and 0·5 a useless test. The cut-off value associated with the best combination of sensitivity and specificity was calculated using differential positive rates [=sensitivity–(1–specificity); Ward 1986]. For pairwise comparisons the Mann-Whitney U test (β-OHB) or Student's *t* test (other variables) were employed. Significance was set at P<0·05.

## RESULTS

β-OHB measurements were non-normally distributed (Ranbut: P<0·001; Precision Xtra: P=0·004) and nonparametric tests were applied. The other variables were normally distributed.

### Analytic evaluation of the ketone meter

Although the statistical analysis of accuracy yielded very good correlation between the ketone meter and the laboratory method (r=0·971; P<0·001) the results differed significantly (Ranbut: median 0·56 mmol/L, range 0·04 to 13·8 mmol/L; Precision Xtra: median 0·4 mmol/L, range 0 to 5·97 mmol/L; P<0·001, Fig 1). At β-OHB concentrations >6 mmol/L measured with the laboratory method [n=9; β-OHB: 8·5 mmol/L (6·08 to 13·8)] the ketone meter values were associated with a significant negative bias [–3·3 mmol/L (–0·78 to –8·97)] and no correlation could be identified (r=–0·05; P=0·898). The intra-day coefficient of variation was 29·4% at low (mean 0·265 mmol/L ±0·078), 5·2% at medium (mean 2·38 mmol/L ±0·123) and 6% at high (4·645 mmol/L ±0·279) concentrations.

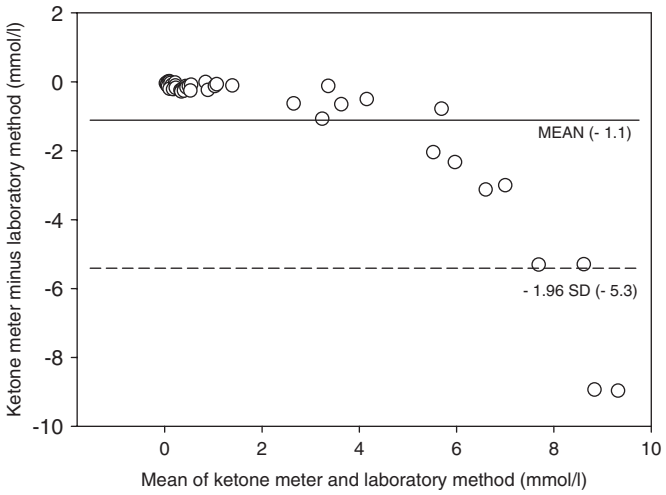


**FIG 1. A Bland-Altman difference plot of β-hydroxybutyrate measurements using a point-of-care ketone meter (Precision Xtra) and a laboratory method from 43 cats. The solid line represents the mean difference, and the dotted line represents the limit of agreement (mean −1·96 sd)**

**Table 1. Demographic and biochemical characteristics of the study population**

| Characteristics | Diabetic ketoacidaemia | Other | P value |
|---|---|---|---|
| Number of admissions | 48 | 169 | |
| Number of cats | 35 | 129 | |
| Age (years) | 9·1 (3) | 10·6 (3·4) | 0·007 |
| Gender (male:female ratio) | 3·8:1 | 1·8:1 | 0·057 |
| Glucose (mmol/L) | 20·3 (7·7) | 18·8 (8) | 0·284 |
| β-Hydroxybutyrate (mmol/L) | 5·25 (0·3 to 6) | 0·8 (0 to 6) | <0·001 |
| Venous pH | 7·12 (0·08) | 7·34 (0·08) | <0·001 |
| Bicarbonate (mmol/L) | 10·2 (2·2) | 21·5 (3·8) | <0·001 |
| Anion gap (mmol/L) | 32·9 (6·6) | 19·5 (5·2) | <0·001 |

P-values refer to Mann-Whitney U (β-hydroxybutyrate, given as median/range), Student's *t* (other variables, given as mean/±1 sd) or Chi-square (gender) tests. P<0·05 considered significant

## Diagnostic performance

β-OHB correlated positively with the AG (r=0·620, P<0·001) and negatively with pH (r=−0·375, P<0·001) and $HCO_3^-$ (r=−0·459, P<0·001).

DKA was identified in 48 (22%) of 217 sets of data. Although cats with DKA were significantly younger than the other cats (Table 1), age was not helpful in clinical decision-making [AUC (ROC) 0·65, 95% confidence interval (CI) 0·563 to 0·732].

Cats with DKA had significantly higher β-OHB concentrations (Fig 2). The AUC (ROC) to identify cats with DKA was 0·84 (95% CI 0·787 to 0·859). The cut-off value 2·55 mmol/L yielded a sensitivity of 94% (95% CI 83 to 99%) and a specificity of 68% (95% CI 59 to 74%, differential positive rate: 0·612). The positive and negative likelihood ratio was 2·83 and 0·09, respectively. The cut-off value combining the highest sensitivity and specificity, e.g., with the highest differential positive rate (0·647) was 3·55 mmol/L [sensitivity 90% (95% CI 77 to 97%)/ specificity 75% (95% CI 67 to 80%)]. The positive and negative likelihood ratio was 3·44 and 0·14, respectively. All cats with DKA

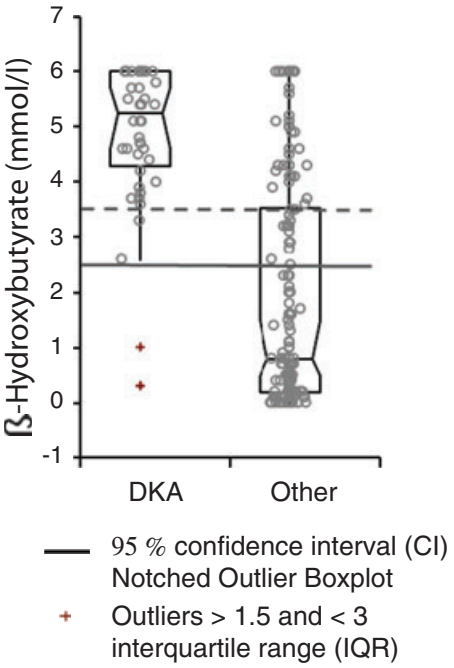


**FIG 2. Scatter and box plots of β-hydroxybutyrate concentrations measured with a hand-held ketone meter in cats with and without diabetic ketoacidaemia (DKA). The two horizontal lines represent the cut-off points 2·55 mmol/L (solid line) and 3·55 mmol/L (dashed line)**

and β-OHB concentrations <3·55 mmol/L (n=3) had coexisting diseases causing additive mixed acid-base disorders (renal failure, n=2; heart failure, n=1). Forty-one samples (19%) collected from 34 cats had β-OHB values >3·55 mmol/L but blood gas analysis did not meet the criteria for DKA as described above. Respiratory compensation ($CO_2$ <34 mmHg) was seen in only six (15%) of these samples. In contrast, 39 (95 %) had an increased [$Cl^-$] gap suggesting coexisting hypochloraemic metabolic alkalosis. Eleven (28%) of these samples were associated with a patient history of vomiting. No significant difference in [$Cl^-$] corrected, [$Cl^-$] gap and β-OHB concentration was found between samples from vomiting and not vomiting cats [P = 0·553, P = 0·098, P = 0·754 (Z=−0·313)]. There was an inverse linear relationship between [$Cl^-$] corrected and β-OHB concentration (r=−0·326, P=0·04).

## DISCUSSION

This study demonstrates that β-OHB measurement using the commercially available point-of-care ketone meter Precision Xtra is a valid tool to rapidly exclude DKA in cats with diabetes mellitus. It is a sensitive and convenient alternative to the current gold standard urine ketone test that semi-quantitatively measures acetoacetate.

At low to moderate concentrations, measurements showed good linear correlation with a reference laboratory method and reproducibility was acceptable. A significant negative bias with differences up to 9 mmol/L was observed at high concentrations. This is in contrast to a study in dogs, where the ketone sensor tended to overestimate β-OHB values (Henderson and

Schlesinger 2010) and possibly limits its usefulness as a monitoring device in the management of feline DKA.

At the cut-off value 2·55 mmol/L the sensitivity of whole blood β-OHB measurements to identify cats with DKA was 94%. Hence at lower concentrations DKA is highly unlikely. The capability to efficiently rule out DKA allows rapid redirection of differential diagnoses in sick diabetic cats and as a consequence earlier treatment of additional diseases that may or may not be related to DM. It also permits better owner communication and helps to decide whether intensive in-hospital treatment is necessary. The suggested cut-off value to "rule out" DKA is very close to the values recommended in dogs (1·9 and 2·8mmol/L; Duarte and others 2002, Di Tommaso and others 2009) and humans (3 to 3·5 mmol/L, Wallace and others 2001, Charles and others 2007, Voulgari and Tentolouris 2010). Increasing the decision concentration to 3·55 mmol/L decreased sensitivity to 90% without markedly improving specificity.

In contrast to studies in humans (Wallace and others 2001, Charles and others 2007, Voulgari and Tentolouris 2010) and dogs (Duarte and others 2002, Di Tommaso and others 2009) that have shown that β-OHB measurements can be used to verify, i.e., rule in DKA, this was not the case in this study. Many cats with β-OHB values above 2·55 or even 3·55 mmol/L did not have the expected theoretical pattern of metabolic acidaemia accompanied by low bicarbonate and a high AG. Possible explanations include differences in respiratory or metabolic compensation, differences in ketone metabolism and differences in the concentration of other acids, e.g., lactate. Whereas hyperventilation with associated respiratory alkalosis was an infrequent finding in these cats, the majority had an increased [$Cl^-$] gap suggesting coexisting hypochloraemic metabolic alkalosis. Although vomiting was likely a contributing factor for chloride losses in some of our cats, vomiting was an inconsistent finding and cannot explain the chloride changes in many of them. The use of an ion-specific electrode and the calculation of the [$Cl^-$] gap reduce the likelihood of "pseudohypochloraemia" caused by hyperlipidaemia or hyperproteinaemia and dilution hypochloraemia. As has been shown for chloride in earlier studies (Christopher and others 1995, Aroch and others, 2011) there was a negative linear association between [$Cl^-$] corrected and β-OHB concentration in this study. Findings in humans suggest that hypochloraemia could be a compensatory mechanism in patients with metabolic acidosis due to accumulation of lactate, ketones and/or other unmeasured acids (Durward and others 2001, Funk and others 2003). In a recent study of feline chronic renal failure, cats in the severe category developed high AG acidosis, but chloride unexpectedly decreased. As with this study vomiting was uncommon and the authors speculated that hypochloraemia likely represents a compensatory adaptive response to metabolic acidosis (Elliott and others 2003b). The results of this study are in line with this hypothesis.

In conclusion, point-of-care β-OHB measurements are reproducible and at low to moderate concentrations sufficiently accurate to be used as a diagnostic tool in feline patients. Concentrations <2·55 mmol/L (sensitivity 94%) exclude DKA and should lead to redirection of differential diagnoses. Due to the common occurrence of mixed acid-base disorders with neutralising effects on pH, especially hypochloraemic metabolic alkalosis superimposed on ketoacidosis, β-OHB measurement cannot replace traditional blood gas analysis and should only be used as a complementary diagnostic measure.

## Conflict of Interest

None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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