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## Evaluation of Precision Xceed® for on-site monitoring of blood β-hydroxybutyric acid and glucose in dairy cows

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## Αξιολόγηση της χρήσης του Precision Xceed® ως μετρητή της συγκέντρωσης του β-υδροξυβουτυρικού οξέος και της γλυκόζης ολικού αίματος των γαλακτοπαραγωγών αγελάδων

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**ABSTRACT.** The aim of the study was to evaluate the usefulness of the Precision Xceed® hand-held meter as an on-site method for determining blood β-hydroxybutyric acid (BHBA) and glucose concentrations, for the diagnosis of subclinical ketosis in dry and lactating dairy cows. A total of 163 clinically healthy Holstein cows (113 lactating, 8-50 days-in-milk; and 50 dry, 10-40 days pre-partum) from 5 farms located around Thessaloniki region, were blood-sampled once, from the jugular vein of each animal, 5 to 8 hours after the start of morning feeding. BHBA was determined in all 163 cows, whereas glucose only in 114 cows (50 dry and 64 lactating cows). These analyses were performed, for each cow, by both laboratory method (in serum) and Precision Xceed® meter (in whole blood, cowside). Using laboratory serum BHBA concentrations  $\geq 1.2$  mmol/L as the cut-off point, 11/163 (6.7%) of the tested cows were considered as subclinically ketotic, whereas raising the cut-off to  $\geq 1.4$  mmol/L, 9/163 (5.5%) cows had subclinical ketosis. All these cows (11 and 9, respectively) were lactating. None of the dry cows had subclinical ketosis at BHBA cut-off of  $\geq 1.4$  mmol/L. One out of the 50 dry cows (2%) and 15/113 (13.3%) lactating cows sampled were classified as subclinically ketotic when the Precision Xceed® meter was set at BHBA concentrations  $\geq 1.2$  mmol/L. Overall, mean BHBA and glucose concentrations were not statistically different ( $P > 0.05$ ) between the two methods. Significant positive correlations were found for

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BHBA (strong correlation:  $r=0.99$ ;  $n=163$ ;  $P<0.01$ ) and glucose (moderate correlation:  $r=0.63$ ;  $n=114$ ;  $P<0.01$ ) concentrations between Precision Xceed® and laboratory results. Precision Xceed® is less accurate for measuring glucose (glucometer) compared to BHBA (ketometer). The low percentage of false positive ( $<0.6\%$ ) and false negative ( $<4\%$ ) indicating that the Precision Xceed® meter is an accurate screening test and its results are highly reliable under field conditions. Precision Xceed® meter was highly sensitive (90.9%) and specific (96.05%) at cut off point of BHBA concentrations  $\geq 1.2$  mmol/L and it had excellent test agreement for detection of subclinical ketosis when using a threshold of blood BHBA  $\geq 1.4$  mmol/L.

**Keywords:** dairy cows, ketometer, glucometer, ketosis

**ΠΕΡΙΛΗΨΗ.** Ο στόχος της παρούσας μελέτης ήταν η εκτίμηση της ακρίβειας και της χρησιμότητας του Precision Xceed®, μιας εύχρηστης φορητής συσκευής για τη μέτρηση της συγκέντρωσης του β-υδροξυβουτυρικού οξέος (BHBA) και της γλυκόζης του ολικού αίματος των γαλακτοπαραγωγών αγελάδων. Η μέτρηση του BHBA είναι απαραίτητη για τη διάγνωση της υποκλινικής κέτωσης των αγελάδων που βρίσκονται στη γαλακτοπαραγωγή ή στην ξηρά περίοδο. Συνολικά χρησιμοποιήθηκαν 163 αγελάδες φυλής Holstein, οι οποίες ήταν κλινικά υγιείς την ημέρα της δειγματοληψίας, από 5 εκτροφές της ευρύτερης περιοχής της Θεσσαλονίκης: οι 113 από αυτές βρίσκονταν στο αρχικό στάδιο γαλακτοπαραγωγής (8-50 ημέρες) και οι υπόλοιπες 50 στο τελικό στάδιο της ξηράς περιόδου (10-40 ημέρες πριν από τον αναμενόμενο τοκετό). Η αιμοληψία έγινε από τη σφαγίτιδα φλέβα, άπαξ σε κάθε αγελάδα, 5 ως 8 ώρες μετά από την πρωινή παράθεση της τροφής. Το BHBA μετρήθηκε και στις 163 αγελάδες, ενώ η γλυκόζη σε 114 (50 στην ξηρά περίοδο και 64 στη γαλακτοπαραγωγή). Η μέτρηση των παραπάνω έγινε, για κάθε ζώο, επιτόπου στις εκτροφές με το Precision Xceed® (στο ολικό αίμα), καθώς και στο Διαγνωστικό Εργαστήριο της Κτηνιατρικής Σχολής Α.Π.Θ. (στον ορό). Όταν για τον καθορισμό της υποκλινικής κέτωσης χρησιμοποιήθηκε ως όριο η συγκέντρωση BHBA ορού αίματος  $\geq 1,2$  mmol/L, 11/163 αγελάδες (6,7%) είχαν υποκλινική κέτωση, ενώ όταν το όριο αυξήθηκε σε  $\geq 1,4$  mmol/L, σε 9/163 αγελάδες (5,5%) προσδιορίστηκε η νόσος. Όλα τα ζώα που εμφάνισαν υποκλινική κέτωση βρίσκονταν στο αρχικό στάδιο της γαλακτοπαραγωγής. Καμία αγελάδα που βρισκόταν στην ξηρά περίοδο δεν εμφάνισε υποκλινική κέτωση, όταν το όριο BHBA για τη νόσο τέθηκε στο  $\geq 1,4$  mmol/L. Χρησιμοποιώντας το Precision Xceed® με όριο για τον καθορισμό της υποκλινικής κέτωσης τη συγκέντρωση BHBA ολικού αίματος  $\geq 1,2$  mmol/L, 1/50 αγελάδες στην ξηρά περίοδο (2%) και 15/113 στη γαλακτοπαραγωγή (13,3%) ταξινομήθηκαν ως περιστατικά υποκλινικής κέτωσης. Συνολικά, οι μέσες συγκεντρώσεις BHBA και γλυκόζης δεν διέφεραν σημαντικά ( $P>0,05$ ) μεταξύ των 2 υπό σύγκριση μεθόδων μέτρησης. Στατιστικά σημαντικές συσχετίσεις βρέθηκαν για το BHBA (ισχυρή συσχέτιση:  $r=0,99$ ;  $n=163$ ;  $P<0,01$ ) και για τη γλυκόζη (μέτρια συσχέτιση:  $r=0,63$ ;  $n=114$ ;  $P<0,01$ ) μεταξύ των αποτελεσμάτων του Precision Xceed® και αυτών των εργαστηριακών μεθόδων. Το Precision Xceed® είναι λιγότερο ακριβές για τη μέτρηση της γλυκόζης (γλυκοζόμετρο) συγκριτικά με την ακρίβεια που παρουσιάζει ως μετρητής του BHBA (κετονόμετρο). Το πολύ χαμηλό ποσοστό ψευδώς θετικών ( $<0,6\%$ ) και ψευδώς αρνητικών ( $<4\%$ ) μετρήσεων ισχυροποιεί την ακρίβεια των αποτελεσμάτων και την αξιοπιστία των μετρήσεων σε συνθήκες κλινικής πράξης. Συμπερασματικά, το Precision Xceed® είναι πολύ ευαίσθητο (90,9%), πολύ ειδικό (96,05%) και έχει άριστη συμφωνία με την εργαστηριακή μέθοδο για τη διάγνωση της υποκλινικής κέτωσης των γαλακτοπαραγωγών αγελάδων, ιδιαίτερα όταν ως όριο για τον καθορισμό της χρησιμοποιείται το επίπεδο BHBA  $\geq 1.4$  mmol/L.

**Λέξεις ευρετηρίασης:** γαλακτοπαραγωγές αγελάδες, φορητός μετρητής, κετονοσώματα, γλυκόζη, κέτωση

## INTRODUCTION

Since the late 1990's, ketosis has emerged as the most important metabolic disease in dairy herds, surpassing ruminal acidosis and milk fever in clinical significance (Oetzel 2007). It typically occurs in dairy cows in early lactation, while it rarely occurs in cattle in late gestation, at which time it resembles pregnancy toxemia of ewes (Merck 2011). Although data concerning pregnancy ketosis's prevalence in dairy cows herds are lacking, it is not considered a significant problem in modern dairies.

Subclinical ketosis (SCK) can be defined objectively as "a condition marked by increased levels of circulating ketone bodies without the presence of clinical signs of ketosis". SCK causes economic losses

in dairy herds directly by decreasing milk production and indirectly by increasing the risk for displaced abomasum and other periparturient diseases (Oetzel 2004).

Serum  $\beta$ -hydroxybutyric acid (BHBA) is the most accurate ketonic body for investigating herds with presumptive ketosis (Oetzel 2004). Researchers use 2 different cut-offs to define SCK: serum BHBA concentration  $\geq 1.2$  mmol/L (Geishauser et al. 1998, Sakha et al. 2007) or  $\geq 1.4$  mmol/L (Duffield 2000, Geishauser et al. 2000, Carrier et al. 2004, Oetzel 2004).

Early and accurate diagnosis of subclinical metabolic disorders, like ketosis, is important for the dairy industry. A variety of cow-side tests (in urine, milk



and, nowadays, blood) are available for ketosis monitoring of dairy herds. However, none of the cow-side tests have perfect sensitivity and specificity compared to blood BHBA measurement in a laboratory (Oetzel 2004). Since determining BHBA in laboratory is costly and time consuming, the evaluation of a reliable on-site test for the determination of BHBA concentration is rewarding. Moreover, measuring on-site blood glucose concentration would, also, contribute in ketosis diagnosis and treatment.

Abbott Laboratories have developed a small hand-held meter (the trade name in Greece is Precision Xceed®, whereas in other countries it is known as Precision Xtra®), for human use, which measures either whole blood BHBA or whole blood glucose, using different strips. As far as we are concerned, no other human glucometer can function as a ketometer as well (i.e. able to measure blood BHBA). There are only few data in the literature concerning the usefulness of this portable meter in diagnosis and decision-making for treatment and prevention of ketosis in dairy cows.

Considering the above, the aim of this study was to evaluate the usefulness of the Precision Xceed® hand-held meter as an on-site method for measuring blood BHBA and glucose concentrations, in order to diagnose ketosis in dry and lactating dairy cows.

## MATERIALS AND METHODS

A total of 163 clinically healthy Holstein cows (3-9 years old, 1-6 lactations), from 5 farms located around the Thessaloniki region, were randomly selected and blood-sampled once. The animals were divided into 2 categories: dry cows (10-40 days pre-calving; 50 animals) and lactating cows (8-50 days in milk - DIM; 113 animals). Sampling was performed from January 2010 until March 2010. The milk yield of the lactating cows ranged from 18.4 to 38.2 kg/d at the sampling period.

BHBA analyses were performed in all 163 cows, whereas glucose was measured in 114 cows (50 dry and 64 lactating cows). Blood samples were collected from the jugular vein of each animal, 5 to 8 hours after the start of morning feeding, by using 18-gauge disposable needles in 10-ml plain glass tubes (BD Vacutainer®) without anticoagulant for serum BHBA and glucose measurement in the laboratory. Before clotting, whole blood BHBA and glucose were measured on-site with

the Precision Xceed® (Abbott, Abbott Diabetes Care Ltd., Oxon, UK) hand-held meter in the farms' office, at a steady room temperature (20°C), following the manufacturer's instructions, within 15 minutes after sampling. After clotting, serum was separated by low speed centrifugation (1600g for 15 minutes) within 15 minutes after sampling, then it was stored in plastic vials and refrigerated at 4°C until analysis. Serum BHBA and glucose concentrations were measured additionally in the Diagnostic Laboratory of the School of Veterinary Medicine. A spectrophotometric kinetic method was used to determine serum BHBA (Gau 1987), whereas a colorimetric spectrophotometric method was used for the determination of glucose concentration (Barham and Trinder 1972).

Since two different cut-off values are used in literature for defining subclinical ketosis, in the present study we have used for analysis both cut-offs. Consequently, lactating cows having serum BHBA concentration  $\geq 1.2$  mmol/L and  $\geq 1.4$  mmol/L were considered as subclinically ketotic, whereas lactating cows having serum BHBA concentration  $< 1.2$  mmol/L and  $< 1.4$  mmol/L, respectively, were considered non-ketotic. On the other hand, there is not any cut-off value for defining subclinical ketosis in dry cows in the accessed literature; consequently, we have used the same with lactational ketosis serum BHBA cut-offs ( $\geq 1.2$  and  $\geq 1.4$  mmol/L) in order to investigate the usefulness of the Precision Xceed® for determining pre-calving blood BHBA. Consequently, dry cows having serum BHBA concentrations  $< 1.2$  mmol/L and  $< 1.4$  mmol/L were, similarly with lactating, considered as non-ketotic.

For statistical analysis, data were entered onto a computerized database and analyzed with the Statistical Program of Social Sciences (SPSS) software for Windows, Version 17.0. Serum BHBA concentrations determined in laboratory were regarded as the gold standard. Correlation coefficients (Pearson's product moment correlation) were calculated between BHBA concentrations in serum measured in the laboratory and values displayed on the hand-held meter test (Precision Xceed®) for whole blood. Means, standard deviations, minimum and maximum values were calculated. Sensitivity, specificity, positive and negative predicted values and their binomial 95% confidence interval (CI95) for the Precision Xceed® hand-held meter at cut-off points (BHBA concen-

**Table 1.** Descriptive statistics of  $\beta$ -hydroxybutyric acid (BHBA) concentrations of dry and lactating dairy cows determined on-site with Precision Xceed® hand-held meter and in laboratory.

Parameter	Method							
	Precision Xceed®				Laboratory			
BHBA (mmol/L)	n	Mean	SD	Min-Max	n	Mean	SD	Min-Max
All cows	163	0.78	0.88	0.20-5.80	163	0.81	0.82	0.28-5.31
Dry cows	50	0.45 <sup>b</sup>	0.18	0.20-1.03	50	0.49 <sup>c</sup>	0.15	0.30-1.01
Lactating cows	113	0.93 <sup>b</sup>	1.01	0.20-5.80	113	0.95 <sup>c</sup>	0.94	0.28-5.31
<i>At BHBA &lt;1.2 mmol/L</i>								
Dry cows	49	0.44 <sup>c</sup>	0.13	0.20-0.80	50	0.49 <sup>f</sup>	0.15	0.30-0.78
Lactating cows	98	0.62 <sup>*c</sup>	0.21	0.20-0.80	102	0.68 <sup>*f</sup>	0.22	0.28-0.74
<i>At BHBA ≥ 1.2 mmol/L</i>								
Dry cows	1	1.30	-	-	0	-	-	-
Lactating cows	15	2.97	1.66	1.20-5.80	11	3.41	1.45	1.20-5.31
<i>At BHBA ≥ 1.4 mmol/L</i>								
Dry cows	0	-	-	-	0	-	-	-
Lactating cows	7	4.12	1.05	2.80-5.80	9	3.88	1.10	2.39-5.31

Mean difference of rows and columns with same letter or \*, respectively, is statistically significant ( $P < 0.05$ ).

tration  $\geq 1.2$  and  $\geq 1.4$  mmol/L) and k-statistics (test agreement) were calculated. Sensitivity was calculated as the proportion of cows with serum BHBA concentrations  $\geq 1.2$  and  $\geq 1.4$  mmol/L correctly diagnosed as positive by the hand-held meter. Specificity was calculated as the proportion of cows with serum BHBA concentrations  $< 1.2$  and  $< 1.4$  mmol/L correctly diagnosed as negative by the hand-held meter test. The positive predicted value was calculated as the proportion of the animals with positive results at the hand-held meter that were truly subclinically ketotic. The negative predictive value was calculated as the proportion of the animals with negative results at the hand-held meter test that were truly non-ketotic. A P value of  $\leq 0.05$  was considered statistically significant.

## RESULTS

Using laboratory serum BHBA concentrations equal or above 1.2 mmol/L as the cut-off point, 11/163 (6.7%) of the tested cows were considered as subclinically ketotic. Raising the cut-off to equal or above 1.4 mmol/L, only 9 out of the 163 cows (5.5%) had subclinical ketosis using the laboratory method. All these (11 and 9, respectively) cows were lactating. One out of the 50 dry cows (2%) and 15/113 lactating cows (13.3%) sampled were classified as subclinically ketotic when the Precision Xceed® meter was used at a cut-off

point of  $> 1.2$  mmol/L. Means, standard deviations, minimum and maximum values of BHBA concentrations determined using the two different methods are shown in Table 1.

BHBA concentrations of all the cows resulted from the Precision Xceed® meter ranged from 0.20 to 5.80 mmol/L, whereas those resulted from the laboratory method varied from 0.28 to 5.31 mmol/L. Overall, the mean whole blood BHBA concentrations resulted from the Precision Xceed® hand-held meter and the laboratory serum BHBA concentrations were not significantly different ( $P > 0.05$ ). However, when cows were stratified according to their production status (dry and lactating), BHBA concentrations were significantly higher ( $P < 0.05$ ) for the lactating compared to dry cows in both methods (laboratory and hand-held meter). None of the dry cows had BHBA concentrations above the cut-off point  $\geq 1.4$  mmol/L.

Glucose concentrations varied from 22-98 mg/dL when determined with the Precision Xceed® meter and from 35-103 mg/dL when determined with the laboratory method. Means, standard deviations, minimum and maximum values of glucose concentrations determined using the two different methods are shown in Table 2. Overall, mean glucose concentration values were significantly higher ( $P < 0.05$ ) with laboratory



**Table 2.** Descriptive statistics of glucose concentrations of dry and lactating dairy cows determined on-site with Precision Xceed® hand-held meter and in laboratory.

Parameter	Method							
	Precision Xceed®				Laboratory			
Glucose (mg/dL)	n	Mean	SD	Min-Max	n	Mean	SD	Min-Max
All cows	114	60.03*	10.88	22-98	114	66.75*	10.19	35-103
Dry cows	50	62.18*	8.99	48-98	50	69.16 <sup>a</sup>	8.30	54-103
Lactating cows	64	58.34*	11.95	22-82	64	64.88 <sup>a</sup>	11.15	35-102
<i>At BHBA &lt;1.2 mmol/L</i>								
Dry cows	49	62.42*	8.90	48-98	50	69.16*	8.30	54-103
Lactating cows	54	60.26*	9.52	48-82	57	66.18*	9.05	51-83
<i>At BHBA ≥ 1.2 mmol/L</i>								
Dry cows	1	50.00	-	-	0	-	-	-
Lactating cows	10	48.00	18.02	22-78	7	54.30	19.87	35-93
<i>At BHBA ≥ 1.4 mmol/L</i>								
Dry cows	0	-	-	-	0	-	-	-
Lactating cows	7	47.00	20.58	22-78	7	54.29	19.87	35-93

Mean difference of rows and columns with same letter or \*, respectively, is statistically significant ( $P < 0.05$ ).

method than that determined with the Precision Xceed® meter. Even when data were stratified according to their production status (dry and lactating), the glucose concentrations remained significantly higher ( $P < 0.05$ ) with the laboratory method than with the Precision Xceed® meter. Moreover, when determined with the laboratory method, the glucose concentrations were significantly higher ( $P < 0.05$ ) for dry than lactating cows (Table 2). When BHBA concentrations were  $< 1.2$  mmol/L, glucose concentrations for lactating and dry cows were significantly higher ( $P < 0.05$ ) when determined with the laboratory method compared to Precision Xceed® meter determination (Table 2). Overall, when determined with the laboratory method, glucose mean concentration was significantly higher ( $P < 0.05$ ) in dry compared to lactating cows.

Analysis of pooled data revealed strongly significant positive correlations (Figure 1 and Figure 2) for BHBA ( $r = 0.99$ ;  $n = 163$ ;  $P < 0.01$ ) and glucose ( $r = 0.63$ ;  $n = 114$ ;  $P < 0.01$ ) concentrations between the Precision Xceed® hand-held meter results and the laboratory results. In addition, a statistically significant negative correlation ( $r = -0.32$ ;  $n = 114$ ;  $P < 0.01$ ) between BHBA and glucose determined with laboratory method was evident, whereas the correlation coefficient between BHBA and glucose determined

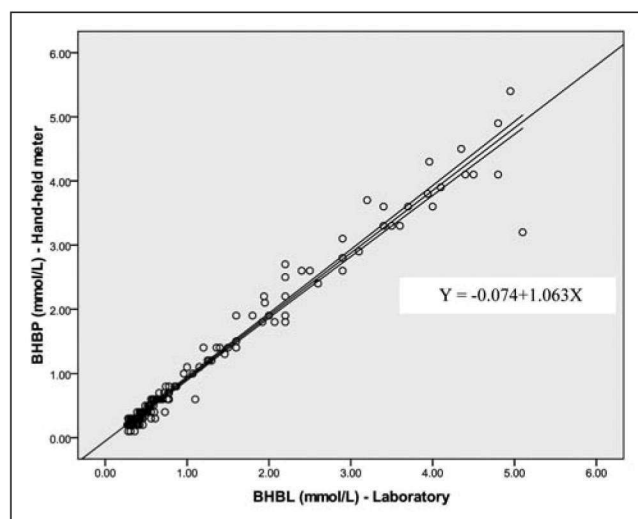
with the hand-held meter was  $-0.44$  ( $n = 114$ ;  $P < 0.001$ ).

Overall, the test performance of the Precision Xceed® hand-held meter at the cut-off points of  $\geq 1.4$  mmol/L was highly sensitive and highly specific relative to serum BHBA for detection of subclinical ketosis. From sensitivity of 90.9% and specificity of 96.05% (cut-off point  $\geq 1.2$  mmol/L), the hand-held meter provided  $< 0.6\%$  false negatives and  $< 4\%$  false positives, respectively. A perfect agreement was revealed, at the cut-off point of  $\geq 1.4$  mmol/L, between the hand-held meter and the serum BHBA for detection of subclinical ketosis in all cows (Table 3).

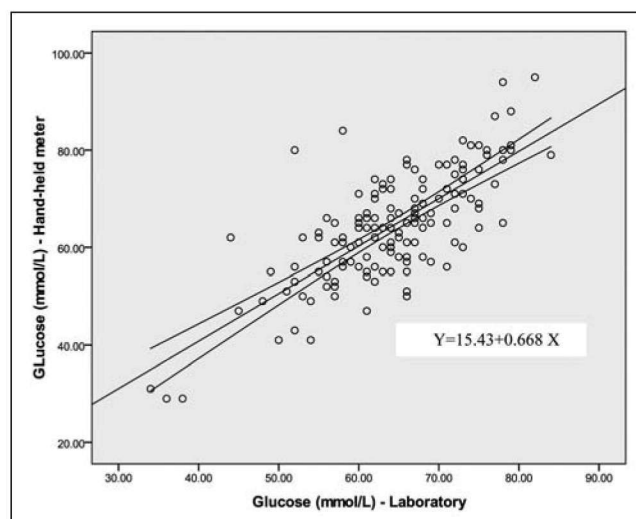
## DISCUSSION

According to the manufacturer's instructions, the suggested for the Precision Xceed® hand-held meter altitude is less than 2,100 m and the suggested reading temperature ranges between 18 and 30 °C for BHBA and 15 and 40 °C for glucose. The altitude where the cow-side measurements in the present study took place was less than 120 m; all readings for BHBA and glucose were done at a room temperature of 22 °C.

There is a diurnal variation of BHBA concentrations in dairy cows, which is related to time after feeding (Eicher et al. 1998, Duffield 2000). In order to capture peak BHBA concentrations, all the blood



**Figure 1.** Correlation of  $\beta$ -hydroxybutyric acid concentrations determined in serum (with laboratory method - BHBL) and whole blood (with Precision Xceed® hand-held meter - BHBP):  $r=0.99$ ;  $n=163$ ;  $P<0.001$ .



**Figure 2.** Correlation of glucose concentrations determined in serum (with laboratory method) and whole blood (with Precision Xceed® hand-held meter):  $r=0.63$ ;  $n=114$ ;  $P<0.001$ .

**Table 3.** Sensitivity, specificity and test agreement (k) for  $\beta$ -hydroxybutyric acid (BHBA) determined with Precision Xceed® hand-held meter in dairy cows.

Precision Xceed® BHBA	BHBA Cut-off (mmol/L)	Sensitivity (CI <sub>95</sub> )	Specificity (CI <sub>95</sub> )	PPV* (CI <sub>95</sub> )	NPV† (CI <sub>95</sub> )	k-statistics
All Cows (n= 163)	$\geq 1.2$	90.9% (57.1-99.5%)	96.1% (91.2-98.4%)	62.5% (35.9-83.7%)	99.3% (95.7-99.9%)	0.72
	$\geq 1.4$	100%	100%	100%	100%	1.00
Lactating cows (n=113)	$\geq 1.2$	90.9% (57.1-99.5%)	95% (88.1-98.1%)	66.7% (38.7-87.1%)	98.9% (93.2-99.9%)	0.74
Dry cows (n= 50)	$\geq 1.2$	98.00% (88.0-99.9%)	NA‡	NA	NA	NA

\*PPV: Positive predicted value

†NPV: Negative predicted value

‡NA: Not applicable

samples in the present research were consistently collected between 5 to 8 hours after the start of the morning feeding. This period would make the possibility to detect subclinical ketosis cases higher. Blood samples for BHBA determination should not be collected from the mammary vein; it is lower in BHBA because the udder tends to extract BHBA but it releases acetoacetic acid (Kronfeld et al. 1968). This ketone body is more stable in blood than acetone or acetoacetate (Tyopponen and Kauppinen 1980). Therefore, blood sampling in the present study was performed with jugular vein puncture of each cow.

The estimated cost of subclinical ketosis is \$78 per

cow, whereas the cost of clinical ketosis is \$145 per cow (Geishauser et al. 2001, Duffield 2003). However, because the subclinical form is more prevalent, its cost at the herd level is much higher (Geishauser et al. 1998, Duffield 2003). Therefore, identification of cows suffering from SCK in the immediate postpartum period at herd level is important to reduce the negative impact of the disease. The prevalence of SCK in a dairy herd can be evaluated by sampling 12 or more cows in early lactation (between 5 and 50 DIM) and determining how many cows have blood BHBA concentrations above 1.4 mmol/L; if more than 10% of the sampled cows have BHBA  $>1.4$  mmol/L, the farm is



considered “at high risk” for ketosis (Oetzel 2004).

Subclinical ketosis's prevalence in dairy herds ranges from 8.9% to 34%, with an average of about 15% (Nielsen et al. 1994, Geishauser et al. 1998, Osborne et al. 2002, Carrier et al. 2004). Evaluating the SCK status of 766 cows in 56 herds, the overall prevalence of SCK was 15%, whereas 34% of these herds had SCK prevalence below 10% (Oetzel 2004). On average, SCK affects 40% of dairy cows at least once during lactation, whereas approximately 5% of them experience clinical ketosis (Geishauser et al. 2001, Duffield 2003). In the present study, 6.7% (11/163) of the tested cows were considered as subclinically ketotic at serum BHBA cut-off concentration of  $\geq 1.2$  mmol/L, whereas elevating the cut-off to 1.4 mmol/L, 5.5% (9/163) of the cows had subclinical ketosis. All these cows were lactating. Since the overall number of tested cows was limited compared to the overall intensively managed dairy cows population in Greece, the above prevalence cannot be considered as representative for the disorders' status in the Greek dairy industry. A thorough investigation in the future is necessary to draw safe conclusions concerning the prevalence of SCK in the Greek dairies.

Overall, in the present study, the mean BHBA concentrations resulted from the Precision Xceed® hand-held meter were similar compared to that arisen from the laboratory method. In other studies in dairy cows, the Precision Xtra® hand-held meter gave significantly higher BHBA values in comparison with the laboratory method (Voyvoda and Erdogan 2010), whereas Iwersen et al. (2009) found slightly lower BHBA values with the hand-held meter.

BHBA concentrations were significantly higher in the lactating compared to dry cows, in both methods, when data were stratified according to the production status (dry or lactating). This is an anticipated finding as it is well-established that lactational ketosis is far more common than pregnancy ketosis (Merck 2011) and the high BHBA concentrations is the main cause of the disease. The same result was, also, evident in non-ketotic cows (serum BHBA  $< 1.2$  mmol/L), when data were stratified according to BHBA concentrations.

A high positive correlation between BHBA measurement with the hand-held meter and laboratory is reported in lactating dairy cows (Jeppesen et al. 2006, Oetzel and McGuirk 2007, Iwersen et al. 2009,

Voyvoda and Erdogan, 2010), in dogs and cats (Hoenig et al. 2008) and in humans (Byrne et al. 2000, Chiu et al. 2002, Noyes et al. 2007). Moreover, a good test agreement is evident between the two measuring methods in cows (Oetzel and McGuirk, 2007, Voyvoda and Erdogan 2010). The present results revealed that the Precision Xceed® had a strong ( $r=0.99$ ), significantly positive correlation, as well as excellent sensitivity and specificity and perfect test agreement with BHBA laboratory measurement, in both pregnant and lactating dairy cows (at a cut-off point  $\geq 1.4$  mmol/L). Consequently, Precision Xceed® can be a very useful tool for on-site monitoring and decision-making for ketosis treatment/prevention in the dairy industry.

Overall, mean glucose concentration resulted from the laboratory method was significantly higher compared to that obtained from the Precision Xceed® hand-held meter. This is in contrast to other results in lactating dairy cows, where glucose results of the hand-held meter were higher compared to those obtained by the laboratory method (Voyvoda and Erdogan 2010). In contrast to BHBA, serum glucose was significantly higher in dry compared to lactating cows, when data were stratified according to the production status (dry or lactating). This is an anticipated result, since lack of energy is the major implicating mechanism of type I ketosis (spontaneous or underfeeding ketosis) (Herdt 2000); as previously mentioned, ketosis is much more prevalent in early lactation than dry period.

The correlation of glucose was moderate ( $r=0.63$ ), significant and positive between the laboratory method and the hand-held meter; however, it was lower than BHBA's correlation. This is in agreement with other results from lactating dairy cows, where the correlation of glucose between the hand-held meter and the laboratory method was lower than that of BHBA (Oetzel and McGuirk 2007, Voyvoda and Erdogan 2010). This hand-held meter is considered less accurate for measuring glucose (glucometer) compared to BHBA (ketometer) in dairy cows (Rollin 2006, Oetzel and McGuirk 2007, Voyvoda and Erdogan 2010). Instead of Precision Xceed®, another portable meter (One Touch Vita) that can only function as a glucometer has been recently found that it can be used in practice to determine glucose concentrations in cows, having very good accuracy (Katsoulos et al. 2011).

The main interest of hand-held glucometers in cattle is found in neonatology and every time a glucose-



containing solution is infused intravenously (Rollin 2006). Although it is not necessary to confirm the hypoglycemia before treating cows with intravenous glucose or an oral glucose precursor, there are situations involving individual sick animals where their glucose status is uncertain and some information about their blood glucose status could guide treatment decisions. These situations could include ketotic (particularly following multiple treatments), any sick cows that are less than about 4 DIM (these cows are often hyperglycemic because they calved so recently), and off-feed, without an obvious cause, cows (Oetzel and McGuirk 2007). In these circumstances, knowledge of the animal's glucose status would be valuable prior to treatment and the Precision Xceed® offers an immediate and quite reliable result. Treating cows with glucose intravenously (or over-treatment with oral glucose precursors) can have negative consequences and it should not be undertaken in cows that are already hyperglycemic (Oetzel and McGuirk 2007).

A statistically significant negative correlation between BHBA and glucose determined with the laboratory method ( $r = -0.32$ ), as well as with the hand-held meter ( $r = -0.44$ ), was revealed. A moderate, but significant, negative correlation between BHBA and glucose concentrations was, also, observed in lactating cows (Sakha et al. 2007, Voyvoda and Erdogan 2010), which is expected, since hypoglycaemia is the major factor that causes subclinical ketosis. However, some dairy cows suffering from type-II ketosis can become ketotic without the coexistence of significant hypoglycaemia (Oetzel 2004).

The low percentage of false positive ( $<0.6\%$ ) and

false negative ( $<4\%$ ) indicating that the Precision Xceed® meter is an accurate screening test and its results are highly reliable under field conditions.

Tests available to clinicians for diagnosing diseases and disorders effectively on-site are rewarding. It is desirable for these tests to: 1) provide rapid, sensitive, specific and reliable results; 2) require small size, easy to use instrumentation; and 3) be inexpensive. The Precision Xceed® hand-held meter meets all these conditions and can be used in place of submitting serum or plasma samples to a laboratory for BHBA analysis. A small drop of whole blood is added to the end of the test strip, which is inserted in the meter, and results are displayed in 5 and 10 seconds, for the glucose and the BHBA, respectively. Less than 1.5  $\mu\text{L}$  of whole blood are required and an important practical issue is that not any additional calibration or adjustment from the human system is necessary in order to obtain the results. The respective blood volume and time for glucose measurement is less than 0.6  $\mu\text{L}$  and 5 seconds, respectively. Furthermore, the cost of the apparatus and strips is affordable and less than laboratory testing. Moreover, sending a blood sample to a laboratory for BHBA analysis requires some processing and it is time-consuming.

## Conclusions

Precision Xceed® hand-held meter seems to be a very useful tool for the practicing veterinarian in cows' medicine. It is highly sensitive, highly specific and it has excellent test agreement for detection of subclinical ketosis, especially when using a threshold of blood BHBA  $\geq 1.4 \text{ mmol/L}$ . ■

## REFERENCES

- Barham D, Trinder P (1972) Improved colour reagent for determination of blood glucose by oxidase system. *Analyst* 97: 142–145.
- Bruss ML (1997) Lipids and ketones. In: *Clinical Biochemistry of Domestic Animals*, 5th ed. Kaneko JJ, Harvey JW, Bruss ML (Eds.), Academic Press, San Diego: pp 83–117.
- Byrne HA, Tieszen KL, Hollis S, Dornan TL, New JP (2000) Evaluation of an electrochemical sensor for measuring blood ketones. *Diabetes Care* 23:500–503.
- Carrier J, Stewart S, Godden S, Fetrow J, Rapnicki P (2004) Evaluation and use of three cowside tests for detection of subclinical ketosis in early postpartum cows. *J Dairy Sci* 87:3725–3735.
- Chiu RW, Ho CS, Tong SF, Ng KF, Lam CW (2002) Evaluation of a new handheld biosensor for point-of-care testing of whole blood beta-hydroxybutyrate concentration. *Hong Kong Med J* 8:172–176.
- Duffield T (2000) Subclinical ketosis in lactating dairy cattle. *Vet Clin N Am – Food A* 16:231–253.
- Duffield T (2003) Minimizing subclinical metabolic diseases. In: *Proceedings of Tri-State Dairy Nutrition Conference*, Fort Wayne, IN, USA: pp 1–14.
- Eicher R, Liesegang A, Bouchard E, Tremblay A (1998) Influence of concentrate feeding frequency and intrinsic factors on diurnal variations of blood metabolites in dairy cows. In: *Proceedings of the 31st Annual Convention of the American Association of Bovine Practitioners*, Rome, GA, USA: pp 198–202.
- Gau N (1987) Acetoacetic acid. In: *Methods in Clinical Chemistry*. Pesce AJ, Kaplan LA (Eds.), CV Mosby Co., St. Louis, MO: pp 97–104.
- Geishauser T, Leslie K, Kelton D, Duffield T (1998) Evaluation of five cowside tests for use with milk to detect subclinical ketosis in dairy cows. *J Dairy Sci* 81:438–443.
- Geishauser T, Leslie K, Tenhag J, Bashiri A (2000) Evaluation of eight cow side ketone tests in milk for detection of subclinical ketosis in dairy cows. *J Dairy Sci* 83:296–299.
- Geishauser T, Leslie K, Kelton D, Duffield T (2001) Monitoring for subclinical ketosis in dairy herds. *Comp Cont Educ Pract Vet* 23:S65–S70.
- Herdth TH (2000) Ruminant adaptation to negative energy balance: Influences on the etiology of ketosis and fatty liver. *Vet Clin N Am – Food A* 16:215–230.
- Hoenig M, Dorfman M, Koenig A (2008) Use of a hand-held meter for the measurement of blood beta-hydroxybutyrate in dogs and cats. *J Vet Emerg Crit Car* 18:86–87.
- Iwersen M, Falkenberg U, Voigtsberger R, Forderung D, Heuwieser W (2009) Evaluation of an electronic cowside test to detect subclinical ketosis in dairy cows. *J Dairy Sci* 92:2618–2624.
- Jeppesen R, Enemark JMD, Enevoldsen C (2006) Ketone body measurement in dairy cows. In: *Proceedings of the 24th World Buiatrics Congress*, Nice, France: Ref: OS 43–2.
- Katsoulos PD, Minas A, Karatzia M, Pourliotis K, Christodouloupoulos G (2011) Evaluation of a portable glucose meter for use in cattle and sheep. *Vet Clin Pathol* 40:245–247.
- Kronfeld DS, Raggi F, Ramberg CF Jr. (1968) Mammary blood flow and ketone metabolism in normal, fasted and ketotic cows. *Am J Physiol* 215: 218–227.
- Merck (2011) Ketosis in Cattle: Introduction. In: Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Whitehouse Station, NJ, USA. <http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bc/80900.htm> [Accessed 2 May 2011].
- Nielen M, Aarts MGA, Jonkers AGM, Wensing T, Schukken YH (1994) Evaluation of two cowside tests for the detection of subclinical ketosis in dairy cows. *Can Vet J* 35:229–232.
- Noyes KJ, Crofton P, Bath LE, Holmes A, Stark L, Oxley CD, Kelnar CJH (2007) Hydroxybutyrate near-patient testing to evaluate a new end-point for intravenous insulin therapy in the treatment of diabetic ketoacidosis in children. *Pediatr Diabetes* 8:150–156.
- Oetzel GR (2004) Monitoring and testing dairy herds for metabolic disease. *Vet Clin N Am – Food A* 20:651–674.
- Oetzel GR (2007) Herd-Level Ketosis – Diagnosis and Risk Factors. In: *Pre-conference Seminar 7C: Dairy Herd Problem Investigation Strategies: Transition Cow Troubleshooting*, 40th Annual Conference of the American Association of Bovine Practitioners, Vancouver, BC, Canada: pp 67–91.
- Oetzel GR, McGuirk S (2007) Fact sheet - Cowside blood BHBA testing with a hand-held “ketometer” - version 4, 3/5/09, School of Veterinary Medicine, University of Wisconsin-Madison, USA. <http://www.vetmed.wisc.edu/dms/fapm/fapmtools/tci/BHBA-Testing-Update-090305.pdf> [Accessed 2 May 2011].
- Osborne TM, Leslie KE, Duffield T, Petersson CS, Ten Hag J, Okada Y (2002) Evaluation of keto-test in urine and milk for the detection of subclinical ketosis in periparturient Holstein dairy cattle. In: *Proceedings of the 35th Conference of the American Association of Bovine Practitioners*, Rome, GA, USA: pp 188–189.
- Rollin F (2006) Tools for a prompt cowside diagnosis: what can be implemented by the bovine practitioner? In: *Proceedings of the 24th World Buiatrics Congress*, Nice, France: pp 75–85.
- Sakha M, Ameri M, Sharifi H, Taheri I (2007) Bovine subclinical ketosis in dairy herds in Iran. *Vet Res Commun* 31:673–679.
- Tyopponen J, Kauppinen K (1980) The stability and automatic determination of ketone bodies in blood samples taken in field conditions. *Acta Vet Scand* 21:55–61.
- Voyvoda H, Erdogan H (2010) Use of a hand-held meter for detecting subclinical ketosis in dairy cows. *Res Vet Sci* 89:344–351.