Clin Chem Lab Med 2010;48(12):1781–1784 © 2010 by Walter de Gruyter • Berlin • New York. DOI 10.1515/CCLM.2010.351

Short Communication

Accuracy of the Precision® point-of-care ketone test examined by liquid chromatography tandem-mass spectrometry (LC-MS/MS) in the same fingerstick sample

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Abstract

Background: The Precision® (Abbott Diabetes Care) point-of-care biosensor test strips are widely used by patients with diabetes and clinical laboratories for measurement of plasma β-hydroxybutyrate (β-HB) concentrations in capillary blood samples obtained by fingerstick. In the literature, this procedure has been validated only against the enzymatic determination of β-HB in venous plasma, i.e., the method to which the Precision® has been calibrated.
Methods: In this study, the Precision® Xceed was compared to a methodologically different and superior procedure: determination of β-HB by liquid chromatography tandem-mass spectrometry (LC-MS/MS) in capillary blood spots. Blood spots were obtained from the same fingerstick sample from out of which Precision® measurements were performed. Linearity was tested by adding varying amounts of standard to an EDTA venous whole blood matrix.
Results: The Precision® was in good agreement with LC-MS/MS within the measuring range of 0.0–6.0 mmol/L (Passing and Bablok regression: slope = 1.20 and no significant intercept, R = 0.97, n = 59). Surprisingly, the Precision® showed non-linearity and full saturation at concentrations above 6.0 mmol/L, which were confirmed by a standard addition experiment. Results obtained at the saturation level varied between 3.0 and 6.5 mmol/L.
Conclusions: The Precision® β-HB test strips demonstrate good comparison with LC-MS/MS. Inter-individual variation around the saturation level, however, is large. Therefore, we advise reporting readings above 3.0 as >3.0 mmol/L. The test is valid for use in the clinically relevant range of 0.0–3.0 mmol/L.
Clin Chem Lab Med 2010;48:1781–4.

Keywords: β-hydroxybutyrate; fingerstick; ketone; liquid chromatography tandem-mass spectrometry; point-of-care; Precision®.

Detection of ketosis is relevant in several conditions, such as alcoholic ketoacidosis, illness or sepsis, dieting, childhood epilepsy and hypoglycemia. However, the most important use of ketone tests is in the detection of potentially fatal ketoacidosis in diabetes mellitus. When insufficient insulin is available to use glucose effectively, the body will begin metabolizing fatty acids as an energy source through the mitochondrial β-oxidation pathway. The end products of this pathway are ketones. The accumulation of ketones in diabetes patients may result in diabetic ketoacidosis (DKA). If left untreated, DKA can lead to coma and death. β-Hydroxybutyrate (β-HB) and acetoacetate are the two main ketone bodies. Acetone, the third ketone body, is present in much lower concentration. The ratio of β-HB to acetoacetate is about 1:1 after a meal, but can rise to 10:1 in DKA (1). The position statement of the American Diabetes Association (ADA) recommends that people with type 1 diabetes be tested for ketone bodies during acute illness or stress, during pregnancy, when blood glucose levels are consistently elevated (>16.7 mmol/L) or when clinical symptoms of ketoacidosis are present (2).

Various studies have shown that blood β-HB measurements are more effective than blood and urine strip methods based on nitroprusside-containing reagents for detecting ketosis and tracking the resolution of DKA (1–4). Nitroprusside reacts with acetoacetate and acetone, but not with β-HB, the predominant ketone body in DKA. Methods that employ nitroprusside-containing reagents are prone to interference from sulfhydryl medications, including the antihypertensive agent captopril. Moreover, nitroprusside test results are qualitative and rely on the ability of the user to differentiate colors. Finally, detection of ketone bodies in

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Received May 28, 2010; accepted June 25, 2010;
previously published online August 24, 2010

urine depends on the ability to urinate, and concentrations often lag 2–4 h behind concentrations in the blood.

Several methods are available to measure β-HB concentrations in blood (5–8). Most are enzymatic methods measuring the concentration of the enantiomer D-β-HB in plasma. The enzyme β-HB dehydrogenase catalyzes the oxidation of D-β-HB to acetoacetate. Concomitant with this oxidation, the cofactor NAD^+ is reduced to NADH and the concomitant change in absorbance can be correlated with the D-β-HB concentration. For five years now, a biosensor test strip for the measurement of β-HB in capillary whole blood samples from fingerstick is on the market for use with the Precision® point-of-care (POC) meter (Abbott Diabetes Care, Alameda, CA, USA) (9). β-HB in the blood specimen reacts with NAD in the presence of β-HB dehydrogenase. This reaction releases electrons that generates a small current proportional to the β-HB concentration. The cells present in the whole blood sample are not lysed, making this a plasma-based measurement. The Precision® test strips have been calibrated to the Ranbut® venous plasma D-β-HB enzymatic assay on the RX Daytona system (Randox Laboratories, Crumlin, Co. Antrim, UK) (5, 6).

Various clinical studies have demonstrated benefits of the Precision® test strips (10–13). Consequently, the Precision® POC meter is widely used by diabetic patients and clinical laboratories for instantaneous measurement of plasma β-HB concentrations in capillary whole blood samples obtained by fingerstick. However, this procedure has been analytically validated only against the enzymatic determination of β-HB in venous plasma, i.e., the method to which the Precision® has been calibrated (10, 13). In the present study, the accuracy of the Precision® ketone test was evaluated by comparing results to a methodologically different and superior procedure: determination of β-HB by liquid chromatography tandem-mass spectrometry (LC-MS/MS) in capillary blood spots. With this new reference method, a fixed volume of whole blood is dried and extracted from filter paper. β-HB is subsequently derivatized and analyzed by liquid chromatography and detected using tandem-mass spectrometry.

All patients with a routine laboratory request for blood ketone analysis over a two-year period were selected for this study. Ethical approval and informed consent were not applicable to the study design. Most of the patients were diabetic patients in an acute care setting. Blood spots were obtained in duplicate from the same fingerstick sample from which the Precision® Xceed measurement was performed. Blood spots were dried and stored at room temperature until they were analyzed for β-HB by LC-MS/MS.

Linearity of the Precision® method was tested by spiking D-β-HB at varying concentrations throughout the range 0–10 mmol/L. In brief, varying amounts of β-HB stock solution and physiological salt solution were added to a freshly obtained EDTA venous blood. The total dilution of the blood matrix was 10% in every sample. To prepare the β-HB stock solution, analytically pure (D,L)-sodium 3-hydroxybutyrate powder (Sigma-Aldrich, Zwijndrecht, The Netherlands) was weighed and dissolved in physiological salt solution. All samples were analyzed with a Precision® Xceed ketone strip and, for comparison, blood spots were obtained in duplicate from each sample for LC-MS/MS analysis. To facilitate comparison, concentrations as measured by LC-MS/MS were divided by a factor of two to reflect concentrations of the D-β-HB enantiomer.

Passing and Bablok (P&B) regression was performed using EP Evaluator Release 8, module CLSI EP9 Method Comparison (David G. Rhoads Associates, Kennett Square, PA, USA).

Results of the method comparison between the Precision® method and blood spot LC-MS/MS for the same fingerstick sample are shown in Figure 1. The number of different patient samples was 68. The Precision® results showed good agreement with LC-MS/MS within the measurement range of 0.0–6.0 mmol/L (P&B regression: slope = 1.20 and no significant intercept; $R = 0.97$, $n = 59$). Surprisingly, the Precision® method showed non-linearity and full saturation at concentrations above 6.0 mmol/L. Inter-individual variation around the saturation level was large, with results varying between 3.0 and 6.5 mmol/L.

Linearity was tested by adding varying concentrations of β-HB throughout the range of 0.0–10.0 mmol/L ($n = 20$). Accurate results were obtained for blood spot LC-MS/MS analysis. The recovery was between 95% and 108%. The Precision® results were compared to LC-MS/MS (Figure 2). Again, the Precision® method showed good agreement with LC-MS/MS within the measurement range of 0.0–6.0 mmol/L (P&B regression: slope = 1.25 and no significant intercept, $R = 0.997$, $n = 18$). The standard addition experiment described here confirmed the presence of saturation at concentrations above 6.0 mmol/L.

Two explanations for the proportional bias within the range of 0.0–6.0 mmol/L that was observed in both experiments (slope = 1.20 and 1.25) can be offered. First, differences in calibration between the Precision® method and LC-MS/MS exist. The Precision® ketone strips are traceable to the Ranbut® venous plasma β-HB enzymatic assay, while the reference LC-MS/MS method is traceable to weighed standard β-HB solutions. The second explanation concerns the nature of the body fluid. The Precision® method is, in principle, a plasma measurement, while blood spot LC-MS/MS uses whole blood as sample material. β-HB concentrations in blood cells may differ from those in plasma. Pilot experiments in our laboratory indeed have demonstrated that β-HB concentrations in plasma are 5%–20% higher than BHB concentrations in whole blood spots as measured by LC-MS/MS (data not shown).

The Precision® ketone measurement shows saturation at concentrations above 6.0 mmol/L which can be attributed to several factors. First, insufficient amounts of reagent on the test strip can cause this effect as enzyme, NAD^+ and other cofactors (e.g., lipids) need to be present in excess. Next, non-linearity of the amperometric detector in the Precision® meter can occur. Finally, the enzyme can be inhibited by the reaction product acetoacetate (15), which when in a ketotic state can be in high concentrations, or the quinoid NADH redox mediator incorporated in the biosensor electrode (9). In the original report (9), Abbott stated that ''diabetics can

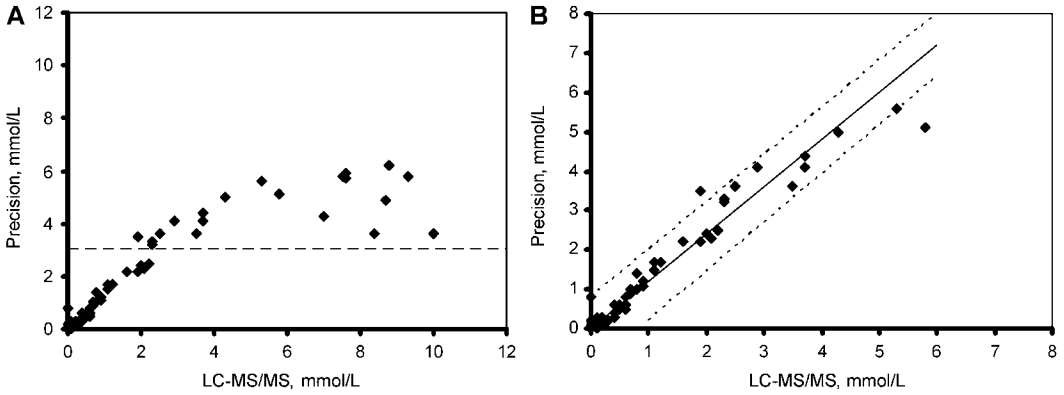

Figure 1 Accuracy of the Precision® ketone test strip examined by comparison to blood spot analysis by LC-MS/MS in the same fingerstick sample.

(A) Complete data set, n = 68. The dashed line represents the advised maximal reporting limit of 3.0 mmol/L. (B) P&B regression was applied within the measurement range of 0.0–6.0 mmol/L. The dashed lines represent the 95% confidence interval (CI) of the regression line. Slope = 1.20 (95% CI: 1.13–1.33) and no significant intercept (95% CI: –0.03–0.10), R = 0.97, n = 59. The LC-MS/MS method was adapted from a previously described method for measurement of lactate and pyruvate (14). Briefly, dried blood spots were extracted with 200 μL methanol/acetic acid (99/1, v/v), after addition of 50 μL internal standard solution ($^{13}C_4$-3-hydroxybutyric acid, Sigma Aldrich, Zwijndrecht, The Netherlands). One hundred microlitre extract was derivatized with 25 μL 3-nitrophenylhydrazine solution (0.14 mol/L in 50% v/v ethanol/water) and 50 μL 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride solution (0.05 mol/L in 1.5% v/v pyridine/ethanol) (60°C, 20 min). After dilution with 8% acetic acid the sample was ready for analysis. Retention of β-HB was obtained using an Acquity UPLC system equipped with an BEH-C18 column (Waters, Milford, MA, USA) and a formic acid/acetonitrile gradient. The Quattro Micro mass spectrometer (Waters, Milford, MA, USA) was operated in ESI-negative mode. MRM transitions used were 238.1 > 194.25 and 242.1 > 196.25 for 3-hydroxybutyric acid and $^{13}C_4$-3-hydroxybutyric acid, respectively.

determine their D-β-HB level with good precision and accuracy over the range of 0.0–6.0 mmol/L''. Surprisingly, in the manual enclosed with the Precision® ketone test strips, a measurement range of 0.0–8.0 mmol/L is described and results >8.0 mmol/L should be reported with the flag, ''HI''. We have never observed this flag when using this device, although some of our samples had BHB concentrations in that range. Furthermore, inter-individual variation around the Precision® saturation level is large, which led us to advise users of the device not to use readings above 3.0 mmol/L, but instead report them as >3.0 mmol/L (Figure 1).

In conclusion, the Precision® β-HB test strip demonstrates good similarity to LC-MS/MS. The test is valid for use in the clinically relevant range (0.0–3.0 mmol/L) (1). We advise users to report readings above 3.0 as >3.0 mmol/L. With this prerequisite, the use of the Precision® β-HB test strip can be a valuable addition in the detection of DKA in an emergency care setting.

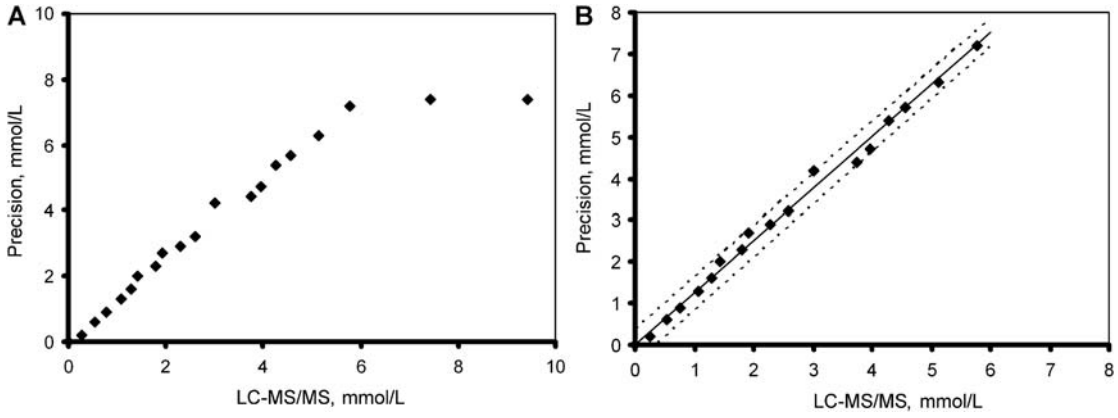

Figure 2 Accuracy of the Precision® ketone test strip examined by comparison to blood spot analysis using LC-MS/MS in a standard addition experiment.

(A) Complete data set, n = 20. (B) P&B regression was applied within the measuring range 0.0–6.0 mmol/L. The dashed lines represent the 95% confidence interval (CI) of the regression line. Slope = 1.25 (95% CI: 1.20–1.30) and no significant intercept (95% CI: –0.14–0.11), R = 0.997, n = 18. Details on the LC-MS/MS method have been described in the legend of Figure 1.

Acknowledgements

We thank Dr. Etienne C.H.J. Michielsen and Dr. Wouter M. Tiel Groenestege, VieCuri Medical Center, for critically reading the manuscript.

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.

References

1. Brink S, Laffel L, Likitmaskul S, Liu L, Maguire AM, Olsen B, et al. ISPAD clinical practice consensus guidelines 2009 compendium: sick day management in children and adolescents with diabetes. Pediatr Diabetes 2009;10(Suppl 12):146–53.
2. Anonymous. ADA position statement: tests of glycemia in diabetes. Diabetes Care 2004;27(Suppl 1):91–3.
3. Guerci B, Tubiana-Rufi N, Bauduceau B, Bresson R, Cuperlier A, Delcroix C, et al. Advantages to using capillary blood beta-hydroxybutyrate determination for the detection and treatment of diabetic ketosis. Diabetes Metab 2005;31:401–6.
4. Sheikh-Ali M, Karon BS, Basu A, Kudva YC, Muller LA, Xu J, et al. Can serum beta-hydroxybutyrate be used to diagnose diabetic ketoacidosis? Diabetes Care 2008;31:643–7.
5. Li PK, Lee JT, Macgillivray MH, Schaeffer PA, Siegel JH. Direct, fixed-time kinetic assays for beta-hydroxybutyrate and acetoacetate with a centrifugal analyzer or a computer-backed spectrophotometer. Clin Chem 1980;26:1713–7.
6. McMurray CH, Blanchflower WJ, Rice DA. Automated kinetic method for D-3-hydroxybutyrate in plasma or serum. Clin Chem 1984;30:421–5.
7. Porter WH, Yao HH, Karounos DG. Laboratory and clinical evaluation of assays for beta-hydroxybutyrate. Am J Clin Pathol 1997;107:353–8.
8. Yamato S, Shinohara K, Nakagawa S, Kubota A, Inamura K, Watanabe G, et al. High-performance liquid chromatography determination of ketone bodies in human plasma by precolumn derivatization with p-nitrobenzene diazonium fluoroborate. Anal Biochem 2009;384:145–50.
9. Forrow NJ, Sanghera GS, Walters SJ, Watkin JL. Development of a commercial amperometric biosensor electrode for the ketone D-3-hydroxybutyrate. Biosens Bioelectron 2005;20:1617–25.
10. Ham MR, Okada P, White PC. Bedside ketone determination in diabetic children with hyperglycemia and ketosis in the acute care setting. Pediatr Diabetes 2004;5:39–43.
11. Harris S, Ng R, Syed H, Hillson R. Near patient blood ketone measurements and their utility in predicting diabetic ketoacidosis. Diabet Med 2005;22:221–4.
12. Naunheim R, Jang TJ, Banet G, Richmond A, McGill J. Point-of-care test identifies diabetic ketoacidosis at triage. Acad Emerg Med 2006;13:683–5.
13. Rewers A, McFann K, Chase HP. Bedside monitoring of blood beta-hydroxybutyrate levels in the management of diabetic ketoacidosis in children. Diabetes Technol Ther 2006;8:671–6.
14. Uran S, Landmark KE, Hjellum G, Skotland T. Quantification of 13C pyruvate and 13C lactate in dog blood by reversed-phase liquid chromatography-electrospray ionization mass spectrometry after derivatization with 3-nitrophenylhydrazine. J Pharm Biomed Anal 2007;44:947–54.
15. Kluger R, Tsui WC. Inhibition of bacterial D-3-hydroxybutyrate dehydrogenase by substrates and substrate analogues. Can J Biochem 1981;59:810–5.