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Accuracy of the Precision® point-of-care ketone test, examined by LC-MS/MS in the same fingerstick sample

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Introduction

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 patients with diabetes mellitus. When insufficient insulin is available to use glucose effectively, the body will metabolize fatty acids as energy source through the mitochondrial beta-oxidation pathway. The endproducts of this pathway are ketones. The accumulation of ketones in patients with diabetes may result in diabetic ketoacidosis (DKA). If left untreated, DKA can lead to coma and death. Beta-hydroxybutyrate (BHB) and acetoacetate are the two main ketone bodies. Acetone, the third ketone body, is present in much lower concentration. The ratio of BHB to acetoacetate is about 1: 1 after a meal, but can rise to 10: 1 in DKA (1). The American Diabetes Association (ADA) position statement recommends measurement of ketone bodies in Type 1 diabetic subjects 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 demonstrated that blood BHB measurement is more effective for detecting ketosis and tracking the resolution of DKA than blood and urine strip methods based on nitroprusside-containing reagents (1-3). Nitroprusside reacts with acetoacetate and acetone but not with BHB, the predominant ketone body in DKA. Methods that employ nitroprusside-containing reagents are prone to interference by 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 urine depends on the ability to pass urine and levels often lag 2 to 4 hours behind the levels in blood.

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Several methods are available to measure BHB concentrations in blood (4-6). Most of them are enzymatic methods measuring the concentration of the enantiomer D-BHB in plasma. The enzyme BHB dehydrogenase catalyzes the oxidation of D-BHB to acetoacetate. Concomitant with this oxidation the cofactor NAD+ is reduced to NADH and the associated change of absorbance can be correlated with the D-BHB concentration. Since five years a biosensor test strip for the measurement of BHB 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) (7). BHB in the blood specimen reacts with NAD in the presence of BHB dehydrogenase. This reaction releases electrons that generate a small current proportional to the BHB concentration. As the cells present in the whole blood sample are not lysed, it is in fact a plasma measurement. The Precision® test strips have been calibrated to the Ranbut® venous plasma D-BHB enzymatic assay on the RX Daytona system (Randox Laboratories, Crumlin, Co. Antrim, UK) (4, 5). Precision of the ketone test strips ranges from 3.1 to 3.8%. The test strips produce accurate results at high altitude, across a hematocrit range of 30-60% and a ketone measurement range of 0.0-8.0 mmol/l and with a minimal sample volume of 1.5 µL. Meter movement, second blood drop application within 30 seconds, various sample application techniques, and numerous drugs and endogenous substances produce no clinically significant effect on the accuracy of the ketone test strips (manual Abbott Diabetes Care).

Clinical studies have demonstrated benefits of the Precision® test strips (8). Consequently, the Precision® POC meter is widely used by diabetes patients and clinical laboratories for instantaneous measurement of plasma BHB concentrations in capillary whole blood samples from fingerstick. However, in literature this procedure has been analytically validated only against the enzymatic determination of BHB in plasma, i.e. the method to which the Precision® has been calibrated (8). In the present study the accuracy of the Precision® ketone test is evaluated by comparing results to a methodologically totally different and superior procedure: determination of BHB by LC-MS/MS in capillary blood spots. In this new method a fixed volume of whole blood is dried and extracted from filter paper. BHB is subsequently derivatized and analyzed by LC-MS/MS.

Methods

All patients with a routine laboratory request for blood ketone analysis in a period of two years (2008-2009) were selected for this study. Most of them were diabetes patients in an acute care setting. Blood spots were obtained in duplicate from the same fingerstick sample in which the Precision® Xceed measurement was performed. Blood spots were dried and stored at room temperature until they were analyzed for BHB by LC-MS/MS.

The LC-MS/MS method was adapted from a previously described method for the measurement of lactate and pyruvate (9). 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). 100 µL 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 BHB was obtained using an Acquity UPLC system equipped with an BEH-C18 column (Waters, Milford, MA, USA) using 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.

Linearity of the Precision® was tested by spiking to varying D-BHB concentrations distributed throughout the range 0-10 mmol/l. In brief, varying amounts of BHB stock solution and physiological salt solution were added to a freshly taken EDTA venous blood matrix. Total dilution of blood matrix was 10% in every sample. To prepare the BHB 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 2 to reflect levels of the enantiomer D-BHB.

Passing & 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 and discussion

Results of the method comparison between Precision® and blood spot LC-MS/MS in the same fingerstick sample are shown in figure 1. The number of different patient samples was 68. The Precision® results were in good agreement with LC-MS/MS within the measuring range 0.0-6.0 mmol/l (P&B regression: r=0.97; slope = 1.20 and no significant intercept; n=59). Surprisingly, the Precision® showed non-linearity and full saturation at concentrations above 6.0 mmol/l. Inter-individual variation around the saturation level was large. Results varied between 3.4 and 6.2 mmol/l.

Linearity was tested by adding varying BHB concentrations distributed throughout the range 0.0-10.0 mmol/l (n=20). Accurate results were obtained for blood spot LC-MS/MS analysis. Calculated recovery was between 95 and 108%. The Precision® results were compared to LC-MS/MS (figure 1). Again, the Precision® was in good agreement with LC-MS/MS within the measuring range 0.0-6.0 mmol/l (P&B regression: r=0.997; slope = 1.25 and no significant intercept; n=18). The standard addition experiment confirmed the presence of a saturation level above 6.0 mmol/l.

Two explanations for the proportional bias within the range 0.0-6.0 mmol/l observed in both experiments (slope = 1.20 and 1.25) can be given. At first, differences in calibration between Precision® and LC-MS/MS exist. The Precision® ketone strips are traceable to

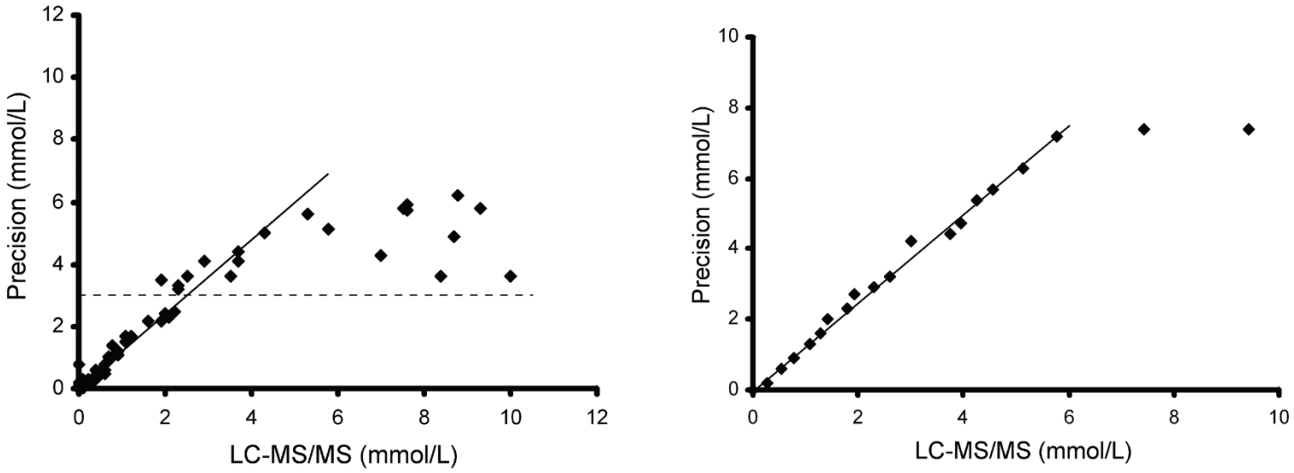

Figure 1. Accuracy of the Precision® ketone test strip as tested by LC-MS/MS. The left panel shows the results of the method comparion of BHB measurement between Precision® and blood spot LC-MS/MS in the same fingerstick sample. Passing & Bablok regression was applied within the measuring range 0.0-6.0 mmol/l. R=0.97; slope = 1.20 (1.13-1.33) and no significant intercept (-0.03-0.10); n=59. The dashed line highlights the advised maximal reporting limit 3.0 mmol/l. The right panel shows the results of the standard addition experiment. Passing & Bablok regression was applied within the measuring range 0.0-6.0 mmol/l. R=0.997; slope = 1.25 (1.20-1.30) and no significant intercept (-0.14-0.11); n=18.

the Ranbut® venous plasma D-BHB enzymatic assay, while the reference LC-MS/MS method is traceable to weighed standard BHB solutions. The second explanation deals with the nature of the body fluid. The Precision® is effectively a plasma measurement, while blood spot LC-MS/MS uses whole blood as sample material. BHB concentrations in blood cells may differ from those in plasma. Pilot experiments in our laboratory indeed have demonstrated that BHB concentrations in plasma are 5-20% higher than BHB concentrations in whole blood (spot) as measured both by LC-MS/MS (data not shown).

The Precision® ketone measurement displays saturation at levels above 6.0 mmol/l which can be attributed to several possible factors. First, insufficient amount of reagents on the test strips. Enzyme, NAD+ and other cofactors (e.g. lipids) have to be present in excess. Next, non-linearity of the amperometric detector in the Precision® meter. Finally, inhibition of the enzyme by the reaction product acetoacetate (10), which can be highly elevated in ketotic state, or by the quinoid NADH redox mediator incorporated in the biosensor electrode (7). In the original scientific report (7) Abbott stated that "diabetics can determine their D-BHB level with good precision and accuracy over the range 0.0-6.0 mmol/l". Surprisingly, in the manual enclosed with the Precision® ketone test strips a measuring range of 0.0-8.0 mmol/l is described and results >8.0 mmol/l should be reported with the flag "HI". This flag has never been observed in our hands, although some samples had BHB concentrations in that range. Furthermore, inter-individual variation around the Precision® saturation level is large, which led us to advise not to use readings above 3.0 mmol/l and report them as >3,0 mmol/l (figure 1).

Conclusion

The Precision® BHB 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). Inter-individual variation around the saturation level, however, was large. Therefore, we advise to report readings above 3.0 as >3.0 mmol/l. With this prerequisite the use of the Precision® BHB test strip can be a valuable addition in the detection of DKA in an emergency care setting.

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