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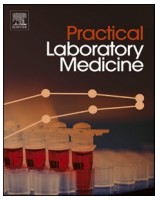

Enzymatic detection of α-hydroxybutyrate, an important marker of insulin resistance, and comparison with LC-MS/MS detection

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ABSTRACT

Aim: The metabolite α-hydroxybutyrate (α-HB) is an important marker of insulin resistance and impaired glucose tolerance allowing to identify patients at risk of developing diabetes and related metabolic disorders before any symptoms become apparent. At present, its exact quantification requires mass spectrometry (LC-MS), which is not compatible with routine laboratory use. Accordingly, a simple enzymatic-based method was assessed and its applicability and measuring accuracy compared with LC-MS was investigated.
Methods: Standards, serum, and plasma samples containing α-HB were prepared with routine procedures and their α-HB contents measured with the XpressGT® enzymatic test kit photometrically or with LC-MS and multiple reaction monitoring.
Results: α-HB detection with XpressGT® yielded highly linear calibration curves and 102 % recovery of stocks added to commercial samples. Stability of the analyte in serum and plasma samples prepared with various anti-coagulants was >90 % after 46 h for several widely used preparations and recovery after 3 freeze-thaw cycles was ≥95 % with these anti-coagulants. A direct comparison of 75 samples indicated very good agreement of α-HB levels determined by both methods, 86 % of XpressGT® samples being within ±20 % of LC-MS values and even 93 % within ±20 % considering only samples above 30 μM concentration.
Conclusion: XpressGT®-based detection of α-HB is an easily applicable method which can be used for accurate and reliable quantification of the metabolite in clinical practice. Routine α-HB determination in patients at risk of developing diabetes would allow early establishment of preventive measures or pharmacological intervention reducing the risk for the onset of serious diabetes-related health problems.

1. Introduction

The worldwide prevalence of diabetes in adults was estimated to be almost 540 million people in 2021 and has been projected to

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increase to 780 million people in 2040 [1]. Diabetes mellitus type 2 (T2D) accounts for up to 95 % of these cases and is characterized by a dysfunction of insulin-producing β-cells and insulin resistance of its target tissues, resulting in dysfunctional blood glucose regulation and long-term health damage [2]. In addition to diagnosed cases of diabetes, a huge number of people remains undiagnosed or has a significant risk of developing diabetes [3,4]. Early onset preventive measures, such as changes in lifestyle, diet and exercise [5] or early pharmacological intervention [6], can dramatically reduce the risk of developing serious diabetic symptoms or delay their occurrence and reduce symptom severity. This also applies to gestational diabetes (GDM), the prevalence of which has increased over the past decades and which is associated with considerable complications at birth and beyond [7,8]. Besides the health burden imposed on the patients affected, T2D is also a major burden on the health system, both monetary and in terms of personnel and infrastructure involved [9]. Accordingly, detection of diabetic markers enabling early prediction and ideally prevention of the disease, as well as diagnosing not yet symptomatic T2D and GDM, in a reliable and scalable manner, is of paramount importance and widely sought-after in clinical research applying, among other methods, metabolomic screenings of non-diabetic and diabetic populations [10–12].

One of the metabolites detected in the course of such screening studies is the intermediate metabolite α-hydroxybutyrate (α-HB, often also referred to as 2-hydroxybutyrate) [12–14], which is linked with dysregulated glucose metabolism. The current model explaining this links assumes that an overload of the TCA cycle due to elevated free fatty acid concentration, as well as increased glycolytic flux due to elevated glucose levels caused by insulin resistance, result in an enhanced NADH/NAD ratio and thereby cause oxidative stress and accumulation of precursors of α-HB generation [10,14]. To produce glutathione to overcome oxidative stress cysteine anabolic pathways are triggered, producing α-ketobutyrate as a byproduct that is subsequently converted to α-HB. Accordingly, α-HB has been shown to be a reliable marker of enhanced diabetic risk, to serve as an early indicator of insulin resistance, β-cell dysfunction, developing hyperglycemia, and also of already established T2D [14–16]. In addition, α-HB levels were found to be altered upon provision of antidiabetic drugs such as the insulin sensitizer pioglitazone or the novel GLP-1 agonist tirzepatide, thereby presenting a measure to monitor therapeutic efficacy of interventions [17,18]. Metabolome profiling has also identified α-HB as a metabolite indicative of metabolically unhealthy obesity in children and adolescents [19] and its elevated levels as a warning signal for the development of GDM [12,20,21]. Importantly, α-HB was not only found elevated in women with established GDM [22], but its level was also reported to be a sensitive predictive marker for women at a risk of GDM in the first trimester [23] and indicative of future complications including postpartum T2D [12]. Overall α-HB appears to fulfill multiple criteria for selection as an early marker for enhanced risk of diabetes and related diseases and as a monitoring parameter in clinical practice that may become increasingly important with newly available drugs for T2D treatment supporting weight loss of patients [18]. However, at present its reliable quantitative determination requires liquid chromatography-mass spectrometry (LC-MS) which is not available in the typical clinical routine lab and thus appears not suitable for routine quantification. To overcome this limitation and make α-HB determination more easily accessible, we have developed the enzyme-based detection kit XpressGT® which is simple to use, fast, reliable, and thus fit to serve as routine quantification method in a clinical setting. Here, we show that this method provides accurate, reproducible, and reliable quantification of serum and plasma α-HB levels both in freshly prepared samples and in samples exposed to prolonged storage or even freeze-thawing cycles. In addition, we report that its α-HB determinations are in excellent agreement with measurements made using LC-MS and allow detecting metabolite levels typical for non-diabetic and pre-diabetic patients and beyond.

2. Material and methods

2.1. Chemicals

(S)-2-hydroxybutyric acid (>97 %, CAS 3347-90-8), sodium (S)-lactate (>98 %, CAS 867-56-1), human plasma (pool, lyophilized, citrate anticoagulant), human serum (male, sterile filtered), and phosphate buffered saline tablets were purchased from Sigma. The latter were dissolved in 200 mL reverse osmosis-generated water with the pH adjusted to 7.40 with NaOH/phosphoric acid. (RS)-2-hydroxybutyric acid sodium salt (>97 %, racemic, CAS 5094-24-6) was from Thermo Fisher Scientific, 2-hydroxybutanoic-d3 acid sodium salt (isotope labeled, racemic, CAS 1219798-97-6) was from Toronto Research chemicals. Sterile human serum and human plasma in sodium citrate, sodium EDTA, and in lithium heparin were obtained from Biotrend CliniSciences Group and were from healthy donors, sterile pooled and gender balanced.

Materials used for liquid chromatography-MS/MS were Elo-Mel isotonic infusion solution from Fresenius Kabi, Graz, Austria, and human serum albumin (200 g/L infusion solution) from Kedrion Biopharma, Austria. Formic acid 99–100 %, AnalaR NORMAPUR®, methanol ≥99,8 %, and HiPerSolv CHROMANORM® were from VWR (Pennsylvania, USA). Purified water was prepared using the Milli Q® Academic water purification System from Millipore GmbH, Vienna, Austria.

2.2. The XpressGT® test kit

The XpressGT® (for research use only) test kit is an enzymatic test kit for the fast and accurate determination of α-HB levels in serum or plasma samples. The kit can be used on spectrophotometric plate readers as well as on automated laboratory analyzers and consists of two reagents that are employed to adjust reaction conditions and neutralize interferences (Reagent 1) and colorimetric determination of the α-HB analyte using an engineered hydroxybutyrate dehydrogenase in conjunction with a tailored colorimetric redox mediator (Reagent 2). For the photometric determination of α-HB, the XpressGT protocol was followed, and all incubation steps were performed in a Tecan M Nano plate reader (Tecan) conditioned at 37 °C with pre-warmed reagents. Serum or plasma samples, standards and controls were thawed on ice and briefly spun down to separate undesired precipitate and cell debris, before 10 µL each were transferred into three separate wells of a preconditioned transparent 96 well plate (Greiner 300 µL, flat bottom) as technical

triplicates. Subsequently, 90 µL of Reagent 1 were added and the mixture incubated for 300 s to pretreat the samples prior to analysis. In the next step, 100 µL of Reagent 2 were added and allowed to mix for 10 s. Immediately thereafter, a kinetic measurement of the 200 µL reaction was performed following the change of the optical absorbance signal at 550 nm for 180 s. These dynamic absorbance changes were then fitted to a linear least squares regression after exporting data to excel and the resulting slope and intercept used to calculate the α-HB concentration according to previously determined calibration data (see Results).

2.3. Preparation of artificial α-HB serum sample set

Besides measuring the native α-HB content of the commercial human serum pool samples, artificial serum stocks were prepared by spiking 160 µL of these serum samples with 40 µL α-HB stock in a randomized fashion (using a number generator), to ultimately obtain 60 samples of this type with final α-HB concentrations ranging between 20 and 80 µM. The spiking aimed at providing samples covering a representative concentration range similar to but expanding the one reported in the study by Varvel et al. for plasma samples from more than 200 patients at increased risk for diabetes [24].

2.4. The LC-MS methodology

An Ultra-high performance liquid chromatography-MS/MS (subsequently simply referred to as LC-MS) method for quantification of α-HB was adapted from the method for β-hydroxybutyrate (β-HB detection) [25]. A stock solution of α-HB was prepared with DL-2-hydroxybutyric sodium salt (racemic) and for the internal standard stock solution isotope labeled 2-hydroxybutanoic-d3 acid sodium salt was used. Both stock solutions were prepared in methanol at a concentration of 1 mg/mL and were stored at - 80 °C in 200 µL tubes until analysis. The calibration standards and quality controls were diluted from the stock solutions with 3 % human serum albumin in isotonic electrolyte solution to obtain the required concentration as detailed in the supplement (Supplemental Tables 1 and 2). 50 µL of sample, standard or quality control were spiked with internal standard and extracted with 200 µL cold methanol. The mixture was vortexed, incubated at 4 °C for 10 min and centrifuged (13000 g; 4 °C; 10 min). The supernatant was then transferred to a 96 well plate and evaporated with compressed air at 37 °C. Before analysis, samples were re-dissolved in 50 µL of methanol/MilliQ water/formic acid (20/80/0.002), v/v. Finally, quantitation of α-HB was conducted with a 1290 Infinity II UHPLC system (Agilent, USA), equipped with an Acquity UPLC BEH C18 (2.1 × 150 mm, 1.7 µm) column (Waters, USA) and coupled to a 6495 B TripleQuad MS (Agilent, USA). The analyte was monitored with negative multi reaction monitoring (MRM).

3. Results

3.1. Calibration

To obtain reference values on the basis of which sample data can be converted to actual α-HB concentrations, a range of calibration standards covering between 10 and 200 µM α-HB were prepared and measured using the XpressGT® test kit. As shown in Fig. 1, this yielded a perfectly linear calibration curve with a coefficient of determination $R^2 > 0.998$ and a slope of 1.548 10^{-4} Abs_{550} min^{-1} µM-1 and an intercept of - 1.524 10^{-4} Abs_{550} min^{-1}.

It should be noted that the enzymatic kit only measures the L-α-HB enantiomer and yielded approximately 50 % of slopes for the racemic mixture containing the same overall concentration of the metabolite (Supplemental Fig. S1).

3.2. Spiking recovery, matrix effects and linearity

Next, multiple aliquots of a commercial plasma pool sample spiked with α-HB at concentrations from 0 to 200 µM were measured

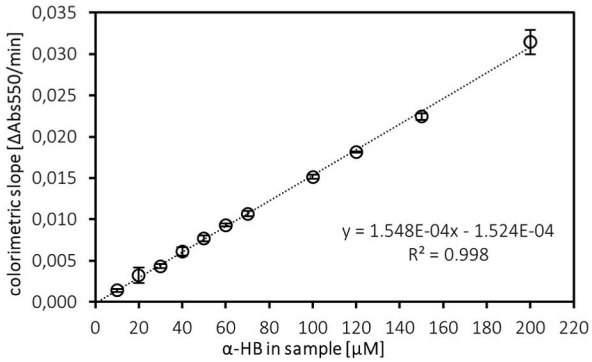

Fig. 1. XpressGT® test kit α-HB calibration curve. The change of absorbance at 550 nm was measured in α-HB standards over 180 s and linear least squares regression was fitted to these data yielding the colorimetric slope plotted on the y-axis. Data points represent means ± SD of 3 technical replicates with SD smaller than symbol size if not visible.

with both the XpressGT® test kit and with LC-MS, and the results of the obtained α-HB concentration values in these samples were plotted against the expected concentration. As shown in Fig. 2, this yielded a highly linear relation between observed and expected (spiked) values for both the test kit and the LC-MS-samples, R^2 of the linear regression plot for the former being 0.999 and for the latter 0.995. The slope of the regression analysis of 1.025 indicated 102 % recovery of added α-HB stocks. Importantly, both methods resulted in a native α-HB concentration of the non-spiked plasma samples of 32.0 ± 3.5 μM, which is well in the range of concentrations previously reported for non-diabetic patients quantified using either LC-MS [13,15] or a targeted, quantitative ^{1}H NMR metabolomics technique [26].

3.3. Specificity and interferences

To evaluate the analytical specificity of the method, alterations reflecting common blood sample defects, clinically relevant blood sample anticoagulants as well as chemical derivatives of α-HB were tested at elevated concentrations. No interfering effects beyond an error margin of ±10 % were observed for serum containing: hemoglobin (1000 < mg/dL), triglycerides (<2000 mg/dL), bilirubin (<120 mg/dL), EDTA (<2.0 %), heparin (<200 U/mL), citrate (<4 %), fluoride (<0.5 %), pyruvate (<3 mM), uric acid (<2.4 mM), creatinine (<1.8 mM), urea (<66 mM), ascorbate (<2.8 mM), L-lactate (<4.0 mM), R-2-hydroxybutyrate (<0.2 mM), 2-hydroxyisobutyrate (<0.2 mM).

3.4. Analytical limits and measurement range

As can be derived from calibration and spiking recovery experiments, the XpressGT® test kit provides a linear relation of signals versus α-HB concentrations up to 200 μM. With the help of plasma blank samples containing no α-HB, a limit of quantification (LOQ), defined as the mean α-HB concentration + 10 SD divided by slope of the standard curve, of 25 μM was reached, spanning an analytical measurement range of 25–200 μM α-HB.

3.5. Sample type and α-HB stability in samples

Commercial samples of serum and plasma containing various types of anti-coagulant were spiked with 100 μM α-HB to increase the signal and then subjected to repeated XpressGT® measurement in triplicates after storage at 4 °C for periods of 0–46 h to assess analyte stability in typical lab conditions. Plotting α-HB concentrations detected in these samples over time of storage indicated that both initial values and the decline with storage time were largely comparable between serum samples and plasma samples prepared with different anti-coagulants (Fig. 3a). Accordingly, in comparison to initial concentrations of spiked samples at 0 h remaining activities detected after 46 h storage were 95 ± 4 % for serum, 87 ± 13 % for plasma EDTA, 98 ± 4 % for plasma Na-citrate and 95 ± 13 % for plasma Li-heparin.

To evaluate sample α-HB stability upon repeated freeze-thawing cycles, which samples may undergo in clinical practice, samples prepared as above were exposed to three consecutive cycles of freezing and thawing before α-HB was again measured using the test kit. As above, all sample types delivered largely comparable α-HB concentration levels following this treatment (serum: 95 ± 4 %, plasma Na-citrate: 96 ± 8 %, plasma Li-heparin: 100 ± 5 %, plasma EDTA: 86 ± 3 % with respect to the initial apparent 0 h concentration) (Fig. 3b). It should be noted that the differences of plasma α-HB concentrations between sample types at zero storage time were within the measurement errors.

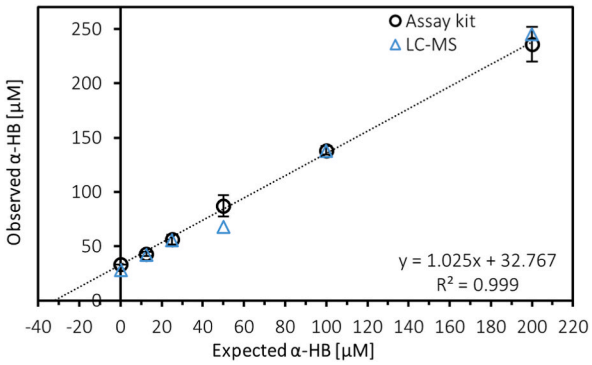

Fig. 2. Comparison between observed and expected α-HB concentration measured with the XpressGT® test kit and LC-MS. Commercial plasma pool samples were spiked with different α-HB concentrations and measured with both methods. Data points represent means ± SD of 3 technical replicates (XpressGT®; open circles) and means of 2 replicates (LC-MS; triangles) and the equation obtained by linear regression analysis of observed versus expected values and the coefficient of determination R^2 is indicated below the data plot.

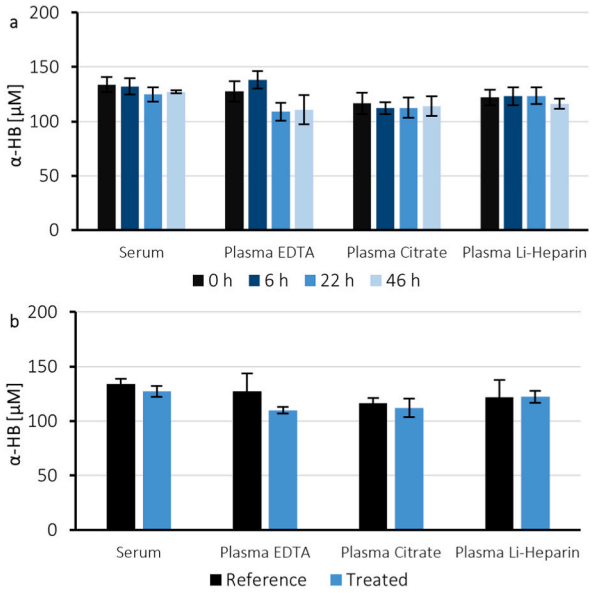

Fig. 3. Stability of α-HB levels during cool storage and after freeze-thawing in different media. Single donor serum and plasma samples spiked with 100 µM α-HB and containing anti-coagulants as indicated were stored at 4 °C and α-HB concentrations measured with the XpressGT® test kit after 0–46 h of storage (a). The same type of samples was exposed to three freeze-thaw cycles and then measured for the remaining α-HB contents (b). Data represent means ± SD of 3 technical replicates.

3.6. *In-assay and between-assay precision*

To evaluate in-assay precision obtained using the test kit, 20 measurements of the 100 µM α-HB standard were carried out creating 20 single datapoints and their concentration calculated based on the calibration curve generated at the start of these experiments. In addition, to obtain an estimate for between-assay precision, 40 independent measurements of sample triplicates were carried out over a period of 10 days and were shared between 2 operators on two photometers.

For in-assay precision (n = 19/20, one sample had to be excluded due to technical problems) an average α-HB concentration of 104.9 ± 3.2 µM (3.1 % relative standard deviation, RSD) was obtained, while the between-assay precision (n = 118/120, two samples excluded) yielded an average value of 106.0 ± 5.5 µM (5.2 % RSD) (Fig. 4). Datasets of both operators were comparable calculating overall means of determinations (105.7 ± 5.5 µM, 106.3 ± 5.5 µM, n = 60) and when calculating averages of triplicates for both operators a value of 105.9 ± 4.0 µM (3.8 % RSD, n = 40/40) was obtained.

3.7. *Accuracy and expected range*

Numerous published studies investigating the predictive value of α-HB levels for diabetes risk have applied LC-MS-methods to

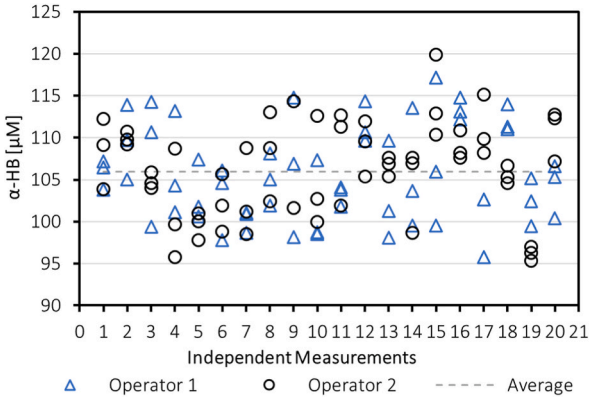

Fig. 4. Between-assay precision (intermediate precision) of α-HB determination using the XpressGT® test kit. 40 independent measurements of sample triplicates of a 100 µM α-HB standard were carried out over a period of 10 days by 2 operators. Data show observed α-HB concentrations for each independent data point for each operator and the overall average value calculated from these data as a dashed line.

quantify the metabolite [13,15,24]. Accordingly, we conducted a direct comparison of XpressGT® measurements and LC-MS (in a specialized lab externally) measurements using the same sample set. To account for differences in the calibration between the labs, that could, e.g., be caused by varying purity of α-HB chemical, a conversion factor § was established using synthetic α-HB calibration samples. To this end, we performed 9 independent measurements on replicates of the measurement standard containing 100 μM α-HB with both methods and obtained values of 111.9 ± 3.3 μM (2.9 % RSD) and 96.7 ± 3.8 μM (4.0 % RSD) with the LC-MS and the XpressGT® method, respectively (Supplemental Fig. S2). The RSD of individual MS measurements of single standards was found to be low but increased when the set of 9 standards was considered, which are technical replicates and originate from a single preparation. Overall, this comparison yielded a conversion factor § of 1.1570 to correct between XpressGT® α-HB and LC-MS-based measurements.

Finally, a sample set of 15 standards (6 spiked plasma pool samples, 9 standards of 100 μM α-HB in buffer) and 60 randomly spiked serum samples were measured with both methods and the concentrations obtained with LC-MS and with the XpressGT® kit after correcting values using § were plotted against each other (Fig. 5). As can be seen in this plot, LC-MS and §-corrected XpressGT® measurements displayed excellent agreement for the sample set ($R^2 = 0.977$) and the linear trendline generated using the 60 datapoints indicated an underestimation bias of 93 % (slope, dotted), by comparison of slopes, for XpressGT® measurements but is likely subject to unequal distribution of the samples over the measurement range.

When considering measurement inaccuracies between LC-MS and XpressGT® measurements at margins of ±20 % (80–120 % of XpressGT® results) 86 % of all samples are situated within these margins. When the set is restricted to samples ≥30 μM α-HB (n = 60/75), 93 % of samples, for >40 μM (n = 45/75) 100 % of samples from the set fall within these margins. For an even stricter criterion of ±10 % (90–110 %), 54 % of all samples fall within these margins, 61 % of samples (≥30 μM) and 73 % of samples (>40 μM), respectively, when an α-HB concentration threshold is defined.

Noteworthy, the calculated average concentration obtained with XpressGT® for the 60 serum samples amounted to 46.2 ± 3.4 μM (4.7 μg/mL), median 41.0 ± 3.4 μM (4.1 μg/mL), which is in good agreement with the median α-HB concentration of 43 μM (4.3 μg/mL, non-fasting) and of 47 μM (4.7 μg/mL, fasting) found in the comprehensive analysis of >90 000 α-HB MS measurements reported by Varvel et al. [24].

4. Discussion

The present study shows that the enzyme-based detection of α-HB with the XpressGT® assay kit allows the accurate and reproducible measurement of the metabolite in serum and plasma samples prepared with routinely used anticoagulants and in samples exposed to prolonged storage at 4 °C or even repeated freeze-thaw-cycles as may occur in clinical practice. The assay produced highly linear calibration curves in the relevant range down to single digit μM concentrations, excellent recovery rates of added stocks and, most importantly, its measurement results were in excellent agreement with those obtained by LC-MS-based α-HB determination.

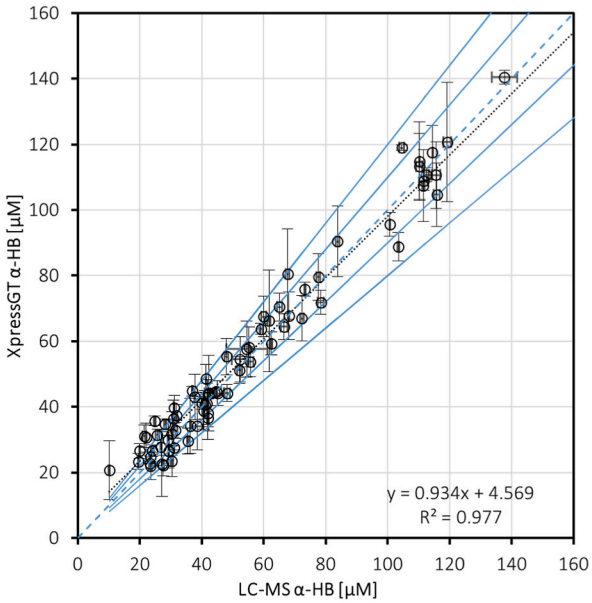

Fig. 5. Comparison of α-HB measurement from standards, spiked plasma and randomized serum samples (n = 75) measured with the XpressGT® test kit and LC-MS. XpressGT®-data were §-corrected and plotted against LC-MS-data yielding a linear relation with slope, intercept and R^2 as indicated above. Overall, an underestimation bias of 93 % (dotted line) for XpressGT® measurements was derived from these measurements. Data represent means ± SD of 2 (LC-MS) and 3 technical replicates (XpressGT®). Blue lines indicate +20 %, +10 %, −10 %, −20 % (from top to bottom) deviation from the ideal match (blue dashed line) between both methods. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Current guidelines to screen for prediabetes and T2D recommend evaluating fasting plasma glucose levels or 2-h plasma glucose values in an oral glucose tolerance test (OGTT), or measuring glycated hemoglobin HbA1c, a long-term indicator of glycemic control [27]. However, the predictive value of such tests was reportedly limited under certain conditions, with many patients displaying normal fasting glucose levels and glucose tolerance but nonetheless progressing to develop T2D [28,29]. Furthermore, HbA1c levels show little sensitivity for detecting subjects at elevated risk for diabetes [30,31], and the determination of these parameters and the exclusion of confounding factors often proof to be cumbersome [32]. Accordingly, more reliable and practical alternatives were looked for in metabolomic studies, identifying α-HB as a biomarker indicative of insulin resistance and impaired glucose regulation [13] and of isolated impaired glucose tolerance [15]. In the former study, biochemical profiling of almost 400 nondiabetic subjects revealed that α-HB levels best distinguished insulin resistant from insulin sensitive subjects assessed by the insulin-surrogate index M_{FFM} [13] and in addition, independently of insulin resistance, identified patients with impaired fasting glycemia or impaired glucose tolerance. The latter study [15], investigating >4000 patients from three European cohorts, reported that α-HB was the metabolite most strongly associated with isolated impaired glucose tolerance, worked as a biomarker independent of age, sex, BMI and fasting glucose and was overall considered suitable to identify impaired glucose tolerance without the necessity to perform an OGTT. In a study on 78 non-diabetic adolescents examined with an OGTT, elevated levels of α-HB both before and during an oral glucose challenge correlated with reduced whole body insulin sensitivity and, strikingly, were predictive of progressive worsening of glucose tolerance and disposition index observed in a follow-up two years later [26]. Finally, α-HB levels were also found to be a sensitive and reliable predictive factor for the development of GDM and the risk of postpartum T2D [12,20–23], completing the overall assessment of α-HB as a useful early marker of enhanced risk for diseases related with abnormal glucose tolerance. Its use as companion diagnostic with other parameters, e.g., to assess the efficacy of pharmacologic interventions, such as the use of novel TD2 and weight loss GLP-1 class drugs [18], and its predictive role for the deterioration of anthropometric parameters beyond diabetes [33] may even extend its value as a biomarker further in the future.

At present, the gold standard for detecting α-HB is by LC-MS-based measurements, which is not routinely available in the typical clinical lab and is also quite labor-intensive and comparatively expensive. In comparison, using the XpressGT® test kit for α-HB determination, a 96-well plate reader was applied, enabling the achievement of a much higher routine throughput as is possible with LC-MS-based measurements. Alternatively, any standard clinical analyzer could be used as well, allowing similarly fast, easily scalable to requirements, and cost-effective measurements in any hospital through implementing the measurement in laboratory routine, reaching all patients where such measurements are deemed useful.

Altogether, given the simplicity, efficiency and reliability of α-HB determinations by use of the XpressGT® assay kit, measurement of this metabolite in serum or plasma samples obtained from patients appear to be easily incorporated into routine clinical practice, measuring the metabolite on typically available clinical analyzers. This would allow determination of this highly valuable biomarker both for screening and monitoring purposes of patients otherwise considered eligible for OGTT or HbA1c measurements, and help to initiate early onset preventive measures [5,6] reducing development and/or progression of T2D and GDM and overall supporting the reduction in costs and human suffering.

5. Conclusions

Serum and plasma levels of α-HB are of predictive value regarding the development of T2D and GDM and can also serve monitoring the disease and its treatment. Since LC-MS, the current gold standard for α-HB detection, is not typically available to clinical routine labs and is relatively time-consuming and expensive, we tested if the enzyme-based XpressGT® detection kit for α-HB is suitable for measuring the metabolite in serum and plasma samples. We found the XpressGT® assay kit to deliver accurate and reproducible measurement in typical routine samples, even when exposed to prolonged storage at 4 °C or repeated freeze-thaw-cycles. Measurements yielded excellently linear calibration curves, full recovery of added stocks and provided results that were in near perfect agreement with LC-MS-based α-HB determinations. Since the XpressGT® assay allows measurements in a multiwell-plate format and on standard clinical analyzers, it can easily be upscaled for high-throughput measurements and could be used for population screening for pre-diabetes, GDM and as companion diagnostic in other contexts yet to be established.

CRediT authorship contribution statement

Beate Steiner: Data curation, Formal analysis, Methodology, Writing – original draft. **Christian Leitner:** Data curation, Formal analysis, Methodology. **David Stadler:** Investigation, Resources. **Eva-Maria Prugger:** Formal analysis, Methodology, Writing – original draft. **Christoph Magnes:** Methodology, Writing – review & editing. **Peter L. Herzog:** Conceptualization, Investigation, Supervision, Writing – review & editing.

Declaration of competing interest

The authors Beate Steiner, Christian Leitner, David Stadler and Peter L. Herzog are employees of DirectSens GmbH. The remaining authors Eva-Maria Prugger and Christoph Magnes declare that there is no conflict of interests regarding the publication of this article.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plabm.2024.e00398.

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