A New Direct Enzymatic Assay for Determination of β-Hydroxybutyrate on Clinical Chemistry Analyzer Platforms

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OBJECTIVE

Determination of β-hydroxybutyrate [HBUT] in serum or plasma can be used for the diagnosis and prognosis of diabetic ketoacidosis, alcoholic ketoacidosis and hypoglycemia. Recently the determination of β-hydroxybutyrate is also used in neurodegenerative diseases, inhibition of adipocyte lipolysis or tumor progression. Identification of ketones [β-hydroxybutyrate (78%), acetone (2%) and acetoacetat (20%)] in serum is exempt from FDA 510(k) premarket notification procedures. The objective of this study was to evaluate CLSI performance of DiaSys β-Hydroxybutyrate 21 FS reagent on Architect c8000™ clinical chemistry analyzer.

MATERIAL AND METHODS

DiaSys β-Hydroxybutyrate 21 FS is a liquid stable ready to use 2-component assay, based on an UV test principle utilizing the β-hydroxybutyrate dehydrogenase dependent conversion of NAD to NADH. The rate of NADH formation is determined by measuring a specific change of absorbance at 340/700 nm, which is directly proportional to the β-hydroxybutyrate concentration in the sample. Evaluated specimen are sera and heparin plasma.

RESULTS

Performance evaluation of DiaSys β-Hydroxybutyrate 21 FS on Architect c8000TM clinical chemistry analyzer revealed a wide linearity range from 0.05 mmol/L up to 8 mmol/L due to a specially adjusted application [Fig.1]. Comparative studies with a commercially available enzymatic colorimetric assay according to CLSI protocol [EP09-A3] show a good correleation of: r = 0.9991; y = 1.012 x + 0.001 mmol/L using regression analysis according to Passing and Bablok [Fig.2]. Moreover, DiaSys β-Hydroxybutyrate 21 FS is highly precise with a repeatability of ≤ 2.06% [Fig. 3] and a total precision according to CLSI protocol [EP05-A2, EP15-A2] of ≤ 3.11% [Fig. 4]. In addition the assay shows a high onboard- and calibration stability of at least 10 weeks [Fig. 5], also interferences like ascorbic acid, hemoglobin or bilirubin are minimized [Fig. 6].

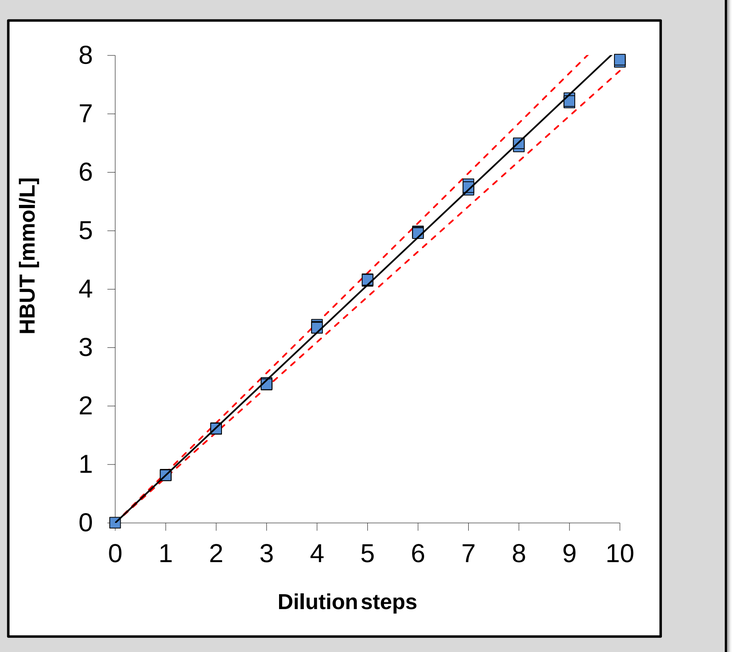


Figure 1: Linearity up to 8 mmol/L hydroxybutyrate

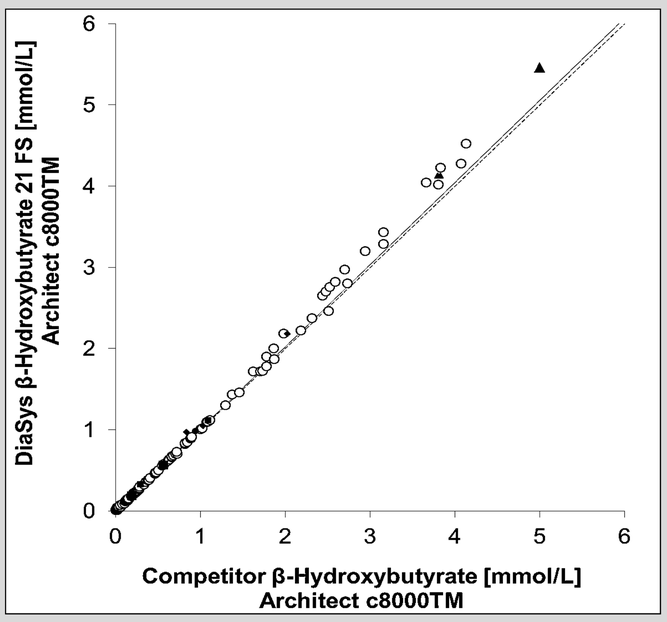
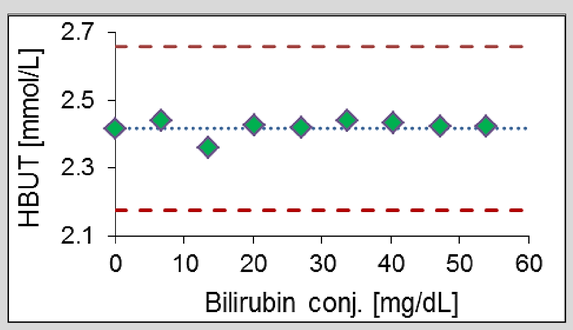
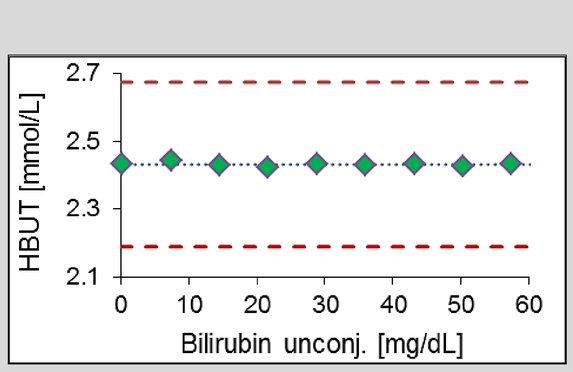
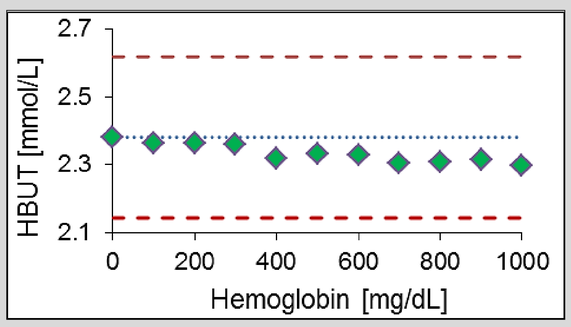


Figure 2: Method comparison
DiaSys against competitor assay







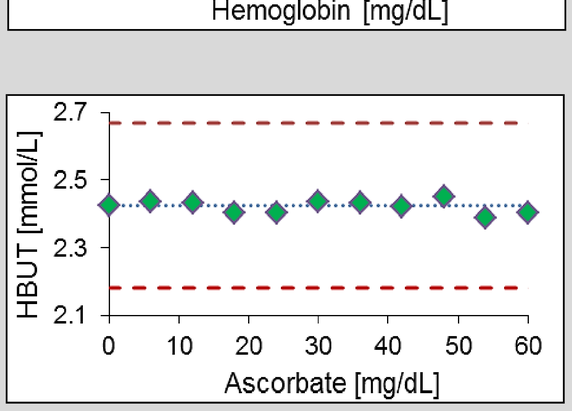


Figure 6: Measurement of interferences

	Sample 1 [mmol/L]	Sample 2 [mmol/L]	Sample 3 [mmol/L]
Mean (n=20)	0.189	0.334	5.64
SD	0.004	0.004	0.016
CV [%]	**2.06**	**1.21**	**0.282**

Figure 3: Precision in series

	Sample 1 [mmol/L]	Sample 2 [mmol/L]	Sample 3 [mmol/L]
Mean (n=84)	0.187	0.329	5.51
SD	0.006	0.005	0.090
CV [%]	**3.11**	**1.67**	**1.64**

Figure 4: Total Precision according to CLSI

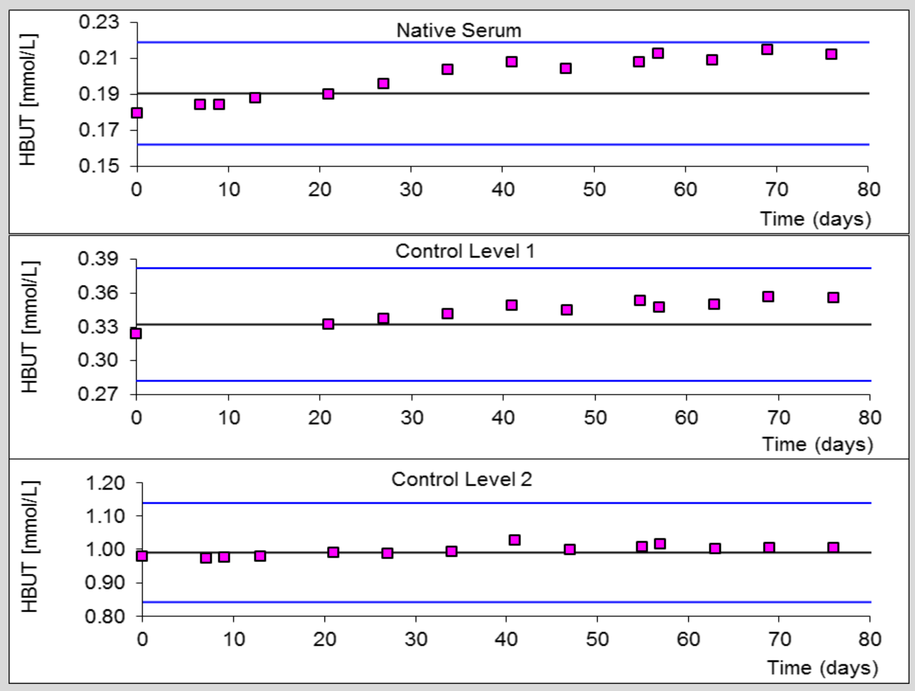


Figure 5 : Onboard- and calibration stability

CONCLUSION

DiaSys β-Hydroxybutyrate 21 FS is well applicable on Architect c8000™ clinical chemistry analyzer and shows outstanding performance especially for linearity and precision. The performance of the test is highly competitive in comparison to already available products in the market and very well suited for diagnosis and monitoring of patients with diabetes to prevent ketoacidosis.

REFERENCES

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