A new high sensitive and specific-kinetic assay for the determination of ß-Hydroxybutyrate on clinical chemistry analyzers

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Objective

ß-Hydroxybutyrate (also known as ß-Hydroxy acid or keto acid, ß-HB) is synthesized in the liver, mainly by oxidation of fatty acids and it is used as source of energy. Serum ß-HB is a key parameter monitored during controlled 24-hour fasts especially to prevent diabetic ketoacidosis. In pediatric patients, the presence or absence of ketonemia/uria is an essential component in the differential diagnosis of inborn errors of metabolism. Evaluation of ß-HB also is essential to detect hypoglycemia, suspected alcohol ingestion or an unexplained increase in the anion gap. Also β-HB has more recently been evaluated for use in neurodegenerative diseases and inhibition of adipocyte lipolysis or tumor progression.
Currently available assays are very expensive and show very low onboard-calibration stability.

Methodology

Here we present a new ready-to-use, two component reagent to measure ß-Hydroxybutyrate on clinical chemistry analyzers (CCA). The new reagent is based on a colorimetric test principle, utilizing the ß-Hydroxybutyrate-dehydrogenase dependent conversion of NAD to NADH. The new reagent shows an excellent performance using two point end. It is also not affected by interferences like ascorbic acid, hemolyzed or icteric samples.

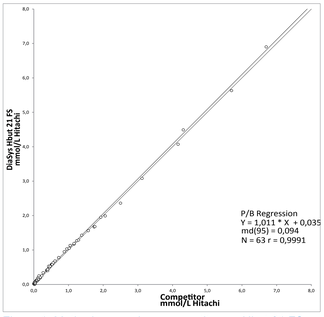

Figure 1: Method comparison competitor vs. Hbut 21 FS

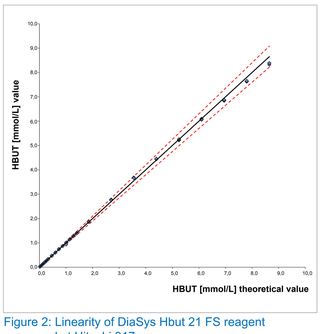

Figure 2: Linearity of DiaSys Hbut 21 FS reagent measured at Hitachi 917

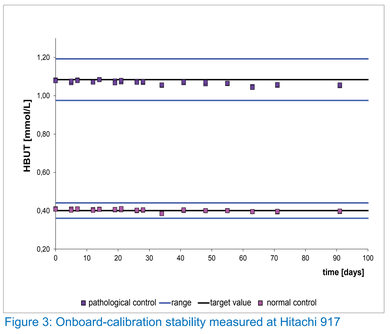

Figure 3: Onboard-calibration stability measured at Hitachi 917

Results

The new test shows a wide linear range from 0,05 to 7,00 mmol/L ß-HB and allows for a robust determination of ß-HB values in serum or plasma samples on routine CCA without prior dilution. Using a Hitachi 917 analyzer system, the test demonstrated an extraordinary < 1 % precision in series at a normal and a pathologic concentration (Table 2). There are no significant interferences at a ß-HB concentration of ~0,20 mmol/L up to 60 mg/dL bilirubin (conjugated and unconjugated), up to 60 mg/dL ascorbic acid , up to 500 mg/dL Hb measured with hemolyzed samples and up to 1400 mg/dL triglyceride (Table 1). The test is performing an extraordinary onboard-calibration stability up to 3 months (Figure 3).

Interferences	Hbut [mmol/L]	Analyte [mg/dL]
Bilirubin conj.	0,25	60
Bilirubin unconj.	0,3	60
Lipid	0,18	1400
Hemolysis	0,24	500
Ascorbic acid	0,26	60

Table 1: Interferences with ± 10% limits measured at Hitachi 917

Precision in series n=20	low level sample	medium level sample
Result [mmol/L]	0,288	0,855
SD [mmol/L]	0,003	0,005
CV [%]	0,96	0,56

Table 2: Precision in series with 2 levels measured at Hitachi 917

Conclusion

The new ß-Hydroxybutyrate 21 FS test is a very sensitive and highly specific method thus very suitable for the diagnosis and monitoring of patients with diabetes to prevent ketoacidosis. The new kinetic test offers a simple, robust and very reliable possibility to diagnose ß-HB in the clinical laboratory routine.

Reference

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