A New Enzymatic Cycling Method for Determination of Total Bile Acids in Serum

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BACKGROUND

Serum total bile acids are a sensitive indicator of liver function and can be used for the diagnosis and prognosis of various liver diseases such as viral hepatitis, intrahepatic cholestasis of pregnancy, liver cancer or toxic hepatic damage. Commercially available assays show limitations regarding the detection of various bile acids. The objective of this study was to compare the new DiaSys total bile acids test with other assays in the market, in particular with regard to the detection of diagnostically relevant primary and secondary bile acids and their conjugates.

METHODOLOGY

The kinetic DiaSys total bile acids test, Bile Acids 21 FS, is based on a cycling reaction which reversibly changes bile acids to an oxidized form, catalyzed by a specific 3-α-hydroxysteroid-dehydrogenase. In this reaction Thio-NAD is reduced to Thio-NADH. In a second reaction step, oxidized bile acids are reduced by the same enzyme with subsequent reduction of NADH to NAD. The rate of Thio-NADH formation is determined by measuring a specific change of absorbance at 405/415 nm, which is directly proportional to the concentration of bile acids in the sample material. Comparative studies were performed on Hitachi 917 with 52 serum samples (mix of native and spiked samples; spiking was done with equal amounts of aqueous glycocholic acid, glycochenodeoxycholic acid, taurocholic acid and taurodesoxycholic acid solutions) [Fig. 1,2]. Data have been evaluated by using regression analysis according to Passing and Bablok. Recovery studies were performed using 50 µM aqueous bile acids solutions [Table 1]. In-series precision studies were performed according to CLSI protocol (EP5-A3) [Table 2].

RESULTS

Bile Acids 21 FS is a liquid stable, 2-component reagent with a sample reagent ratio of 4:270:90 µL (on Hitachi 917). Linearity is given up to 220 µmol/L. The reagent shows good correlation for sera in comparison studies with commercially available methods [Fig. 1,2]. The DiaSys test outperformed the competitors' assays with respect to the recovery (Mean of deviations to 100% = 5.10% vs. 8.12% (competitor A) vs. 23.67% (competitor B)) [Table 1]. Furthermore, Bile Acids 21 FS shows a good in-series precision with a CV of ≤ 2.89% [Table 2].

Recovery in aqueous bile acids solution [50µM]	DiaSys Cycling Method		Competitor A Cycling Method		Competitor B Cycling Method	
	[µmol/L]	[%]	[µmol/L]	[%]	[µmol/L]	[%]
Glycocholic acid	50.3	100.5	49.0	97.9	33.8	67.5
Glycochenodeoxycholic acid	46.0	92.0	52.0	104.0	50.9	101.9
Taurochenodesoxycholic acid	48.0	96.1	42.1	84.3	44.8	89.6
Taurocholic acid	49.6	99.2	44.8	89.7	31.9	63.7
Chenodeoxycholic acid	52.0	104.0	55.8	111.7	54.9	109.8
Cholic acid	53.6	107.3	50.4	100.9	35.8	71.5
Desoxycholic acid	53.9	107.8	55.8	111.7	69.7	139.4
Taurodesoxycholic acid	51.4	102.7	48.0	96.0	59.7	119.5
Lithocholic acid	44.6	89.1	43.7	87.3	32.7	65.4

Table 1: Recovery of primary and secondary bile acids

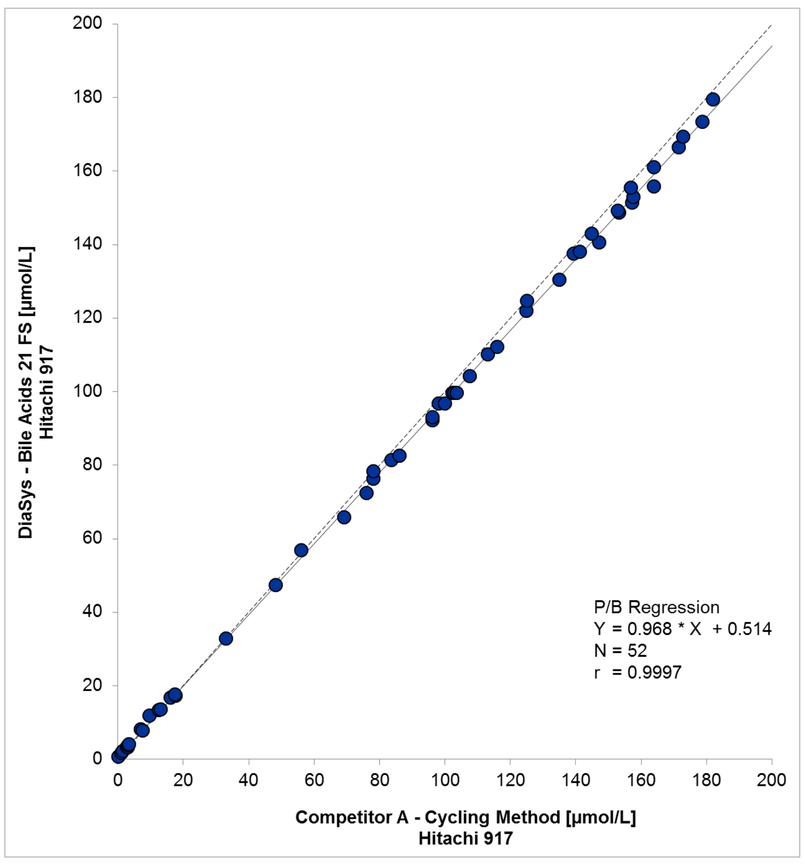

Figure 1: Competitor A - comparison total bile acids

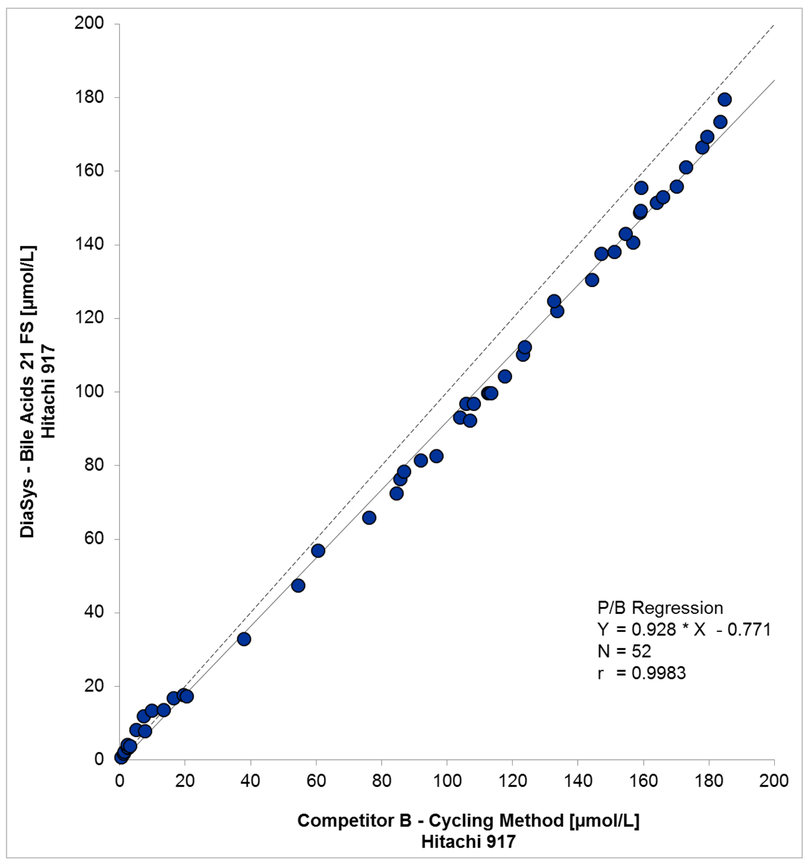

Figure 2: Competitor B - comparison total bile acids

Mean [µmol/L]]	SD [µmol/L]]	CV [%]
7.37	0.21	2.89
16.2	0.27	1.67
36.7	0.42	1.13

Table 2: In-series precision (n=20)

CONCLUSIONS

All results clearly show that Bile Acids 21 FS meets the requirements of a sensitive test to analyze all stages of impaired liver function. Diagnostically relevant primary and secondary bile acids as well as their conjugates are reliably recovered. The performance of the test is highly competitive in comparison to competitor products.

REFERENCES

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