New DiaSys Enzymatic Method for Determination of Total Bile Acids in Serum on BioMajesty® JCA-BM6010/C Clinical Chemistry Analyzer

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Background

Total bile acid (TBA) content is a sensitive marker of liver function for diagnosis and monitoring of various liver diseases [1]. Increased TBA levels are associated with acute and chronic hepatitis, intrahepatic cholestasis of pregnancy (ICP) [2], liver sclerosis, cirrhosis and cancer. Commercially available assays show limitations regarding the detection of clinically relevant primary and secondary bile acids. DiaSys introduces a new liquid-stable, ready-to-use reagent for assessment of all relevant bile acids in a sample offering the possibility to precisely cover all stages of liver diseases.

Methodology

The enzymatic Total bile acids 21 FS test is based on a specific 3-α-hydroxysteroid dehydrogenase cycling reaction converting Thio-NAD 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 a change of absorbance at 410/596 nm, which is directly proportional to the concentration of bile acids in the sample material [3]. Recovery studies on various primary and secondary bile acids were performed using 50 µM aqueous bile acids solutions [Table 3]. In-series precision studies have been performed according to CLSI protocol (EP5-A3) [Table 1, 2]. Comparative studies were performed with 100 serum samples. Data have been evaluated by using regression analysis according to Passing and Bablok.

RESULTS

Linearity of the new Total bile acids 21 FS test is up to 220 µmol/L [Fig. 1]. Total bile acids 21 FS shows a very good in-series precision with a CV of ≤ 0.83% (at 10 µmol/L). Furthermore, method comparison of Total bile acids 21 FS with 100 native samples against a competitor test demonstrated excellent correlation [r = 0.9963; Passing/Bablok: y = 1.026 x + 0.211 µmol/L] [Fig. 2]. Calibration stability of up to 28 days has been achieved for the new test on BioMajesty® JCA-BM6010/C which is a decisive advantage over competition [Fig. 3].

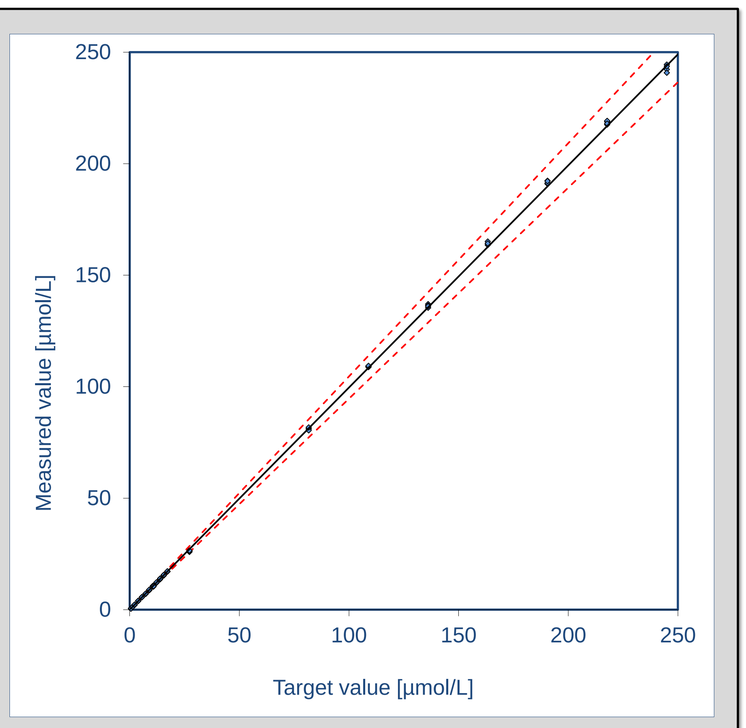

Figure 1: Linearity up to 220 µmol/L total bile acids

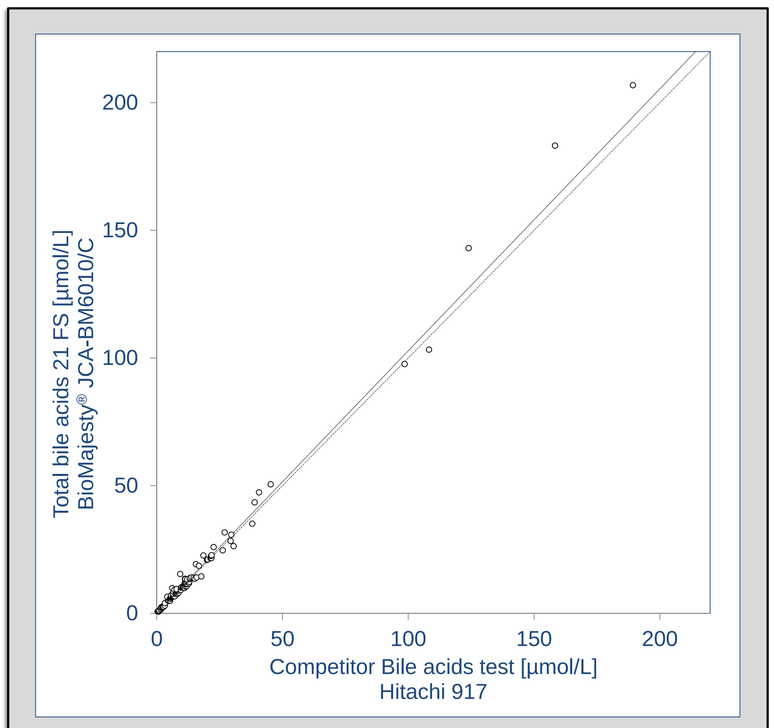

Figure 2: Method comparison DiaSys vs. competitor

	Sample 1 [µmol/L]	Sample 2 [µmol/L]	Sample 3 [µmol/L]
Mean (n=20)	5.41	10.2	199
SD	0.129	0.085	1.19
CV [%]	**2.38**	**0.83**	**0.60**

Table 1: Precision in series

	Sample 1 [µmol/L]	Sample 2 [µmol/L]	Sample 3 [µmol/L]
Mean (n=84)	5.39	10.4	201
SD	0.076	0.159	1.64
CV [%]	**1.42**	**1.54**	**0.82**

Table 2: Precision day-to-day

Recovery in aqueous bile acids [50 µM]	DiaSys Cycling Method		Competitor A Cycling Method		Competitor B Cycling Method	
	[µmol/L]	[%]	[µmol/L]	[%]	[µmol/L]	[%]
Glycocholic acid	53.4	107	36.2	72	38.9	78
Glycochenodeoxycholic acid	48.9	98	49.4	99	67.0	134
Taurochenodeoxycholic acid	51.8	104	48.5	97	57.2	114
Taurocholic acid	50.3	101	34.0	68	34.6	69
Chenodeoxycholic acid	53.5	107	51.9	104	75.8	152
Cholic acid	51.6	103	35.6	71	38.4	77
Deoxycholic acid	50.1	100	61.8	124	74.6	149
Taurodeoxycholic acid	47.3	95	56.5	113	56.8	114
Lithocholic acid	48.4	97	-	-	-	-

Table 3: Recovery studies on various primary and secondary bile acids

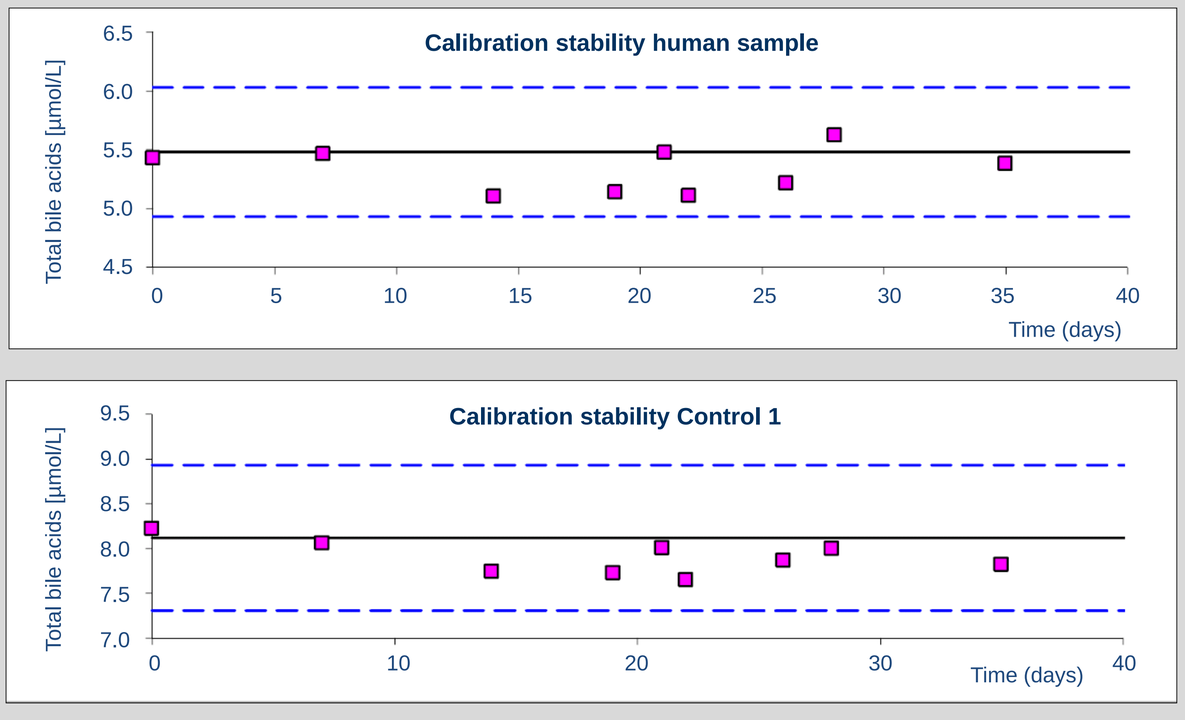

Figure 3 : Calibration stability

CONCLUSION

DiaSys Total bile acids 21 FS is well applicable on BioMajesty® JCA-BM6010/C clinical chemistry analyzer and shows outstanding performance, especially for the recovery of all diagnostically relevant primary and secondary bile acids. The performance of the test is highly competitive in comparison to commercially available products and very well suited for assessment of liver functions as well as for diagnosis and monitoring of ICP.

REFERENCES

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3. Zhang GH et al. An enzymatic cycling method for the determination of serum total bile acids with recombinant 3alpha-hydroxysteroid dehydrogenase. Biochemical and biophysical research communications. 2005;326: 87–92.