New application of the DiaSys Total bile acids 21 FS enzymatic cycling method for the determination of total bile acids in human stool samples on the automated respons® 910 clinical chemistry analyzer

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BACKGROUND

Determination of total bile acids (TBA) in human stool plays an increasingly important role in diagnosis of bile acid malabsorption (BAM), which might lead to various gastrointestinal tract disorders, such as irritable bowel syndrome with diarrhea (IBS-D), bile acid diarrhea (BAD) or Crohn's disease. Approximately 25 – 50% of IBS-D patients and 1% of the Western population suffer from BAD. The gold standard for BAD diagnosis, the 7-days selenotaurohomocholic acid retention test (^{75}SeHCAT), as well as other currently applied methods display significant disadvantages [1-2]. Therefore, DiaSys introduced the innovative, liquid-stable reagent Total bile acids 21 FS for determining TBA in human stool samples on the clinical chemistry analyzer respons® 910.

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 405/600 nm, which is directly proportional to the concentration of bile acids in the sample material [3]. The validation of the new application was performed according to Clinical and Laboratory Standards Institute (CLSI) protocols and following the new European regulation for *in vitro* diagnostics (2017/745). All results were obtained in 1:100 diluted stool extracts. Comparative studies were performed with 122 stool extracts and evaluated by Passing and Bablok regression analysis.

RESULTS

Linearity of Total bile acids 21 FS test in stool extracts is up to 130 µmol/L [**Fig. 1**]. Total bile acids 21 FS shows a very good repeatability [CV of ≤ 2.06%; **Tab. 1**] and good total precision [CV ≤ 4.09%; **Tab. 2**] . Method comparison against a competitor test (n=122) demonstrated good correlation [r = 0.988; y = 1.22 x + 1.89 µmol/L; **Fig. 2**]. There were no significant interferences with common endogenous stool substances. [**Fig. 3**]. Compared to a competitor assay, the DiaSys test displayed more precise recovery values of clinically relevant bile acids [**Tab. 4**].

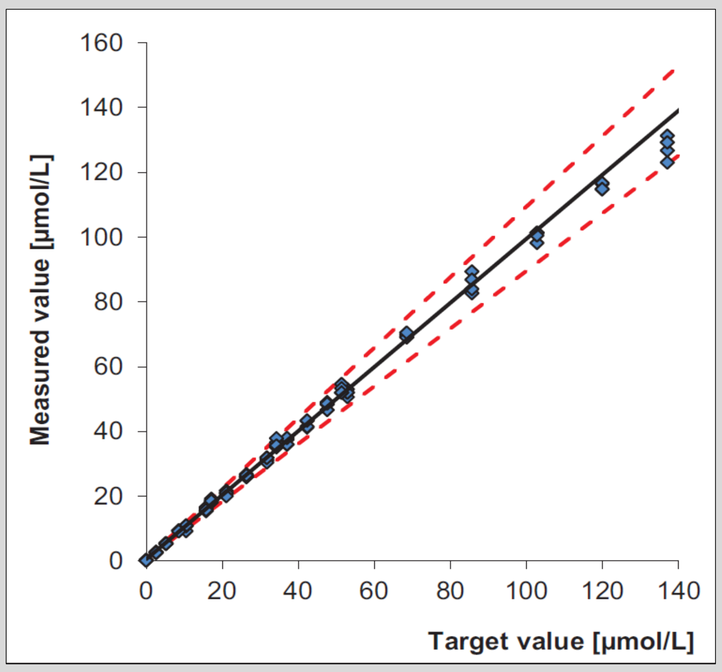

Figure 1: Linearity up to 130 µmol/L total bile acids [Dilution 1:100]

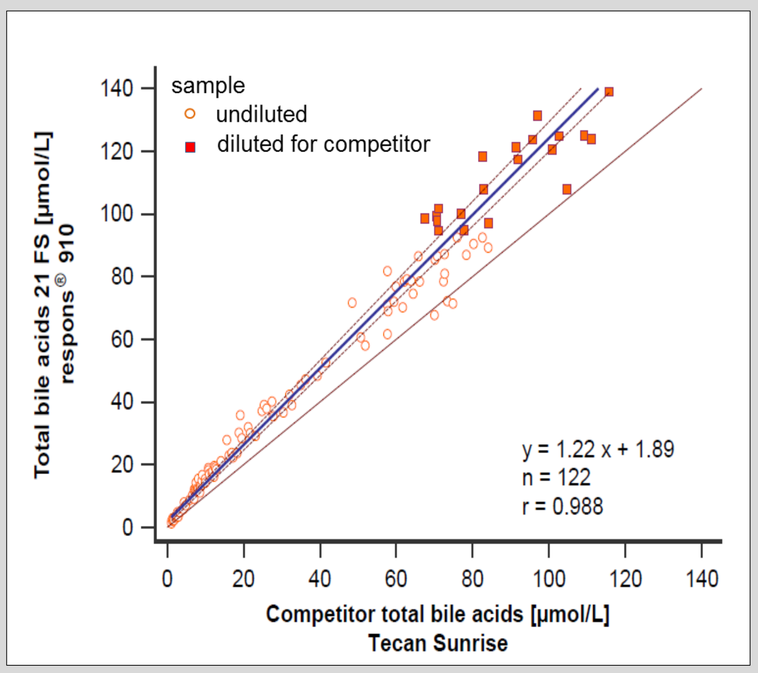

Figure 2: Method comparison (CLSI) DiaSys vs. competitor. [Dilution 1:100]*

	Sample 1	Sample 2	Sample 3
Mean (n=20)	14.7	70.8	115
SD	0.222	0.770	2.37
CV [%]	**1.50**	**1.09**	**2.06**

Table 1: Repeatability CLSI [Dilution 1:100]

	Sample 1	Sample 2	Sample 3
Mean (n=80)	14.8	72.5	120
SD	0.607	2.54	4.13
CV [%]	**4.09**	**3.51**	**3.44**

Table 2: Total precision (CLSI) [Dilution 1:100]

Bile Acid	Reference values RP-E SI-MS/MS	Recovery values DiaSys Total bile acids 21 FS	Recovery values Competitor assay
		[%]	[%]
Cholic acid	38.9	101	105
Chenodeoxycholic acid	39.0	96	142
Deoxycholic acid	45.4	77	144
Glycocholic acid	52.9	80	83
Glycochenodeoxycholic acid	48.8	78	116
Glycoursodeoxycholic acid	60.9	99	65
Taurocholic acid	34.0	123	123
Taurochenodeoxycholic acid	47.0	88	121
Taurodeoxycholic acid	46.8	93	162
Ursodeoxycholic acid	54.8	101	67
Glycodeoxycholic acid	55.1	73	133

Table 3: Recovery studies on various primary and secondary bile acids (LC/MS)

Interfering Substance	Interferences in stool sample	Analyte concentration
Ascorbic acid	1.20 mg/dL 1.20 mg/dL	30.5 86.6
Bilirubin conjugated	0.74 mg/dL 0.74 mg/dL	31.8 91.8
Bilirubin unconjugated	0.68 mg/dL 0.68 mg/dL	31.1 90.3
Hemoglobin	120 mg/L 120 mg/L	30.8 88.9
Immunoglobulin A	30 mg/L 30 mg/L	30.7 87.6
Lipemia	240 mg/L 240 mg/L	30.1 84.7

Table 4 : Minimized interferences from endogenous substances

*Red squares: sample dilution for competitor test due to limited measuring range

CONCLUSION

The successful application of DiaSys Total bile acids 21 FS for stool samples on the automated analyzer respons® 910 combined with the remarkable performance data, makes this innovative approach cost-efficient and timesaving. The widely used and uncomplicated method ensures a convenient and fast workflow for TBA determination in stool in routine laboratories.

REFERENCES

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