Supplementary Table S1. Reagents for the quantitative *in vitro* determination of total bile acids in stool samples.

	Total bile acids 21 FS (stool)	**IDK® Bile Acids Photometric Kit (stool)**
Manufacturer	DiaSys Diagnostic Systems GmbH (Holzheim, Germany)	Immundiagnostik AG (Bensheim, Germany)
Catalog number	1 2238 99 10 921	K 7878W
Lot numbers	30970 (Lot 1), 32893 (Lot 2), 31665 (Lot 3)	R1 211-068, R2 211-069
Matrix	Stool	Stool
Calibrator	TruCal TBA (40.3 µmol/L; Cat #1 2240 99 10 037)	STD, ready-to-use (0; 6; 12; 24; 48; 96 µmol/L)
Controls	TruLab N (8.3 µmol/L; Cat #5 9000 99 10 061)	CTRL1, ready-to-use (9.7 µmol/L)
	TruLab P (32.4 µmol/L; Cat #5 9000 99 10 061)	CTRL2, ready-to-use (30 µmol/L)

Supplementary Table S2. Performance characteristics (stool samples).

		Total bile acids 21 FS (stool)	IDK® Bile Acids Photometric Kit (stool)
Linearity[1]		3.5 – 130 µmol/L	1.89 – 54.83 µmol/L
Detection capability[2]	Limit of blank (LoB)	0.16 µmol/L	0.34 µmol/L
	Limit of detection (LoD)	1 µmol/L	1.89 µmol/L
	Limit of quantification (LoQ)	3.5 µmol/L	1.89 µmol/L
Within-run precision[3] (Repeatability)	Samples	n = 20	n = 40
	Levels (concentration)	3 (14.7, 70.8, 115 µmol/L)	2 (8.13, 25.86 µmol/L)
	CVs	1.1 – 2.1 %	3.4 – 5.2 %
Within-laboratory precision[3] (Total precision)	Samples	n = 80	n.d.
	Levels (concentration)	3 (14.8, 72.5, 120 µmol/L)	
	CVs	3.44 – 4.09 %	
Reproducibility[3]	Samples	n = 75	n = 22
	Levels (concentration)	3 (15.0, 74.2, 125 µmol/L)	2 (8.46, 27.04 µmol/L)
	CV	3.01 – 4.08 %	5.4 – 5.9 %

		Total bile acids 21 FS (stool)	IDK® Bile Acids Photometric Kit (stool)
Interferences[4] ≤10% up to	Ascorbic acid	1.2 mg/dL	no interference observed
	Bilirubin	0.74 mg/dL (conjugated) 0.68 mg/dL (unconjugated)	no interference observed
	Hemoglobin	12 mg/dL	no interference observed
	Immunoglobulin A (IgA)	3 mg/dL	n.d.
	Triglycerides	24 mg/dL	no interference observed
Stability[5]	Onboard (with chimneys)	14 days	n.d.
	Calibration (with chimneys)	7 days	n.d.

Evaluated according to the following CLSI guidelines: [1] EP06 (2nd Edition), [2] EP17 (2nd Edition), [3] EP05 (3rd Edition), [4] EP07 (3rd Edition), and [5] EP25.

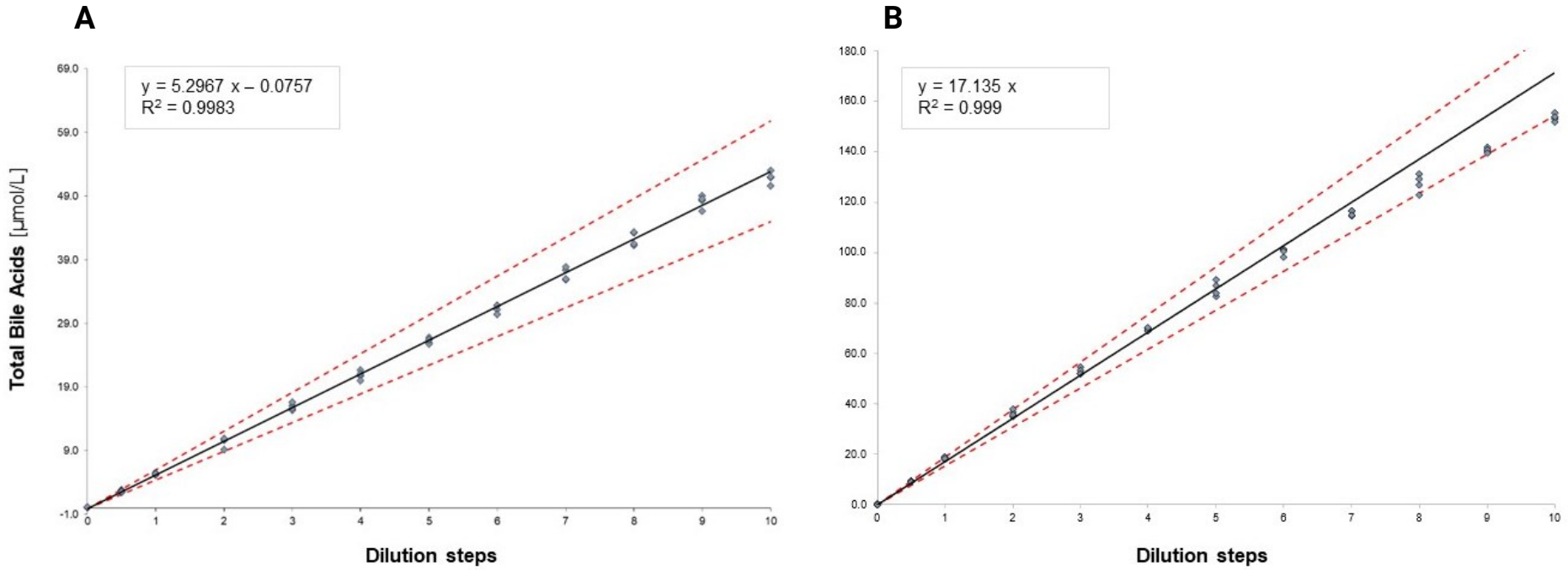
Supplementary Table S3. Application parameters (stool samples).

	BioMajesty® JCA-BM6010/C Automated random access analyzer (up to 1.200 tests/hour)		**respons® 910** Benchtop clinical chemistry analyzer (up to 150 tests/hour)	**respons® 940** Automated random access analyzer (up to 640 tests/hour)	**Tecan Sunrise™** Microplate reader
Assay	IDK® Bile Acids	DiaSys TBA 21 FS	DiaSys TBA 21 FS	DiaSys TBA 21 FS	IDK® Bile Acids
Reagent 1	90 µL	90 µL	180 µL	180 µL	150 µL
Reagent 2	30 µL	30 µL	60 µL	60 µL	50 µL
Sample volume	3 µL	1.3 µL	5 µL	3 µL	10 µL
Reading cycles	25 / 32	25 / 32	29 / 40	33 / 44	n/a
Wavelength	410 / 596 nm	410 / 596 nm	405 / 600 nm	415 / 600 nm	405 nm / 620 nm

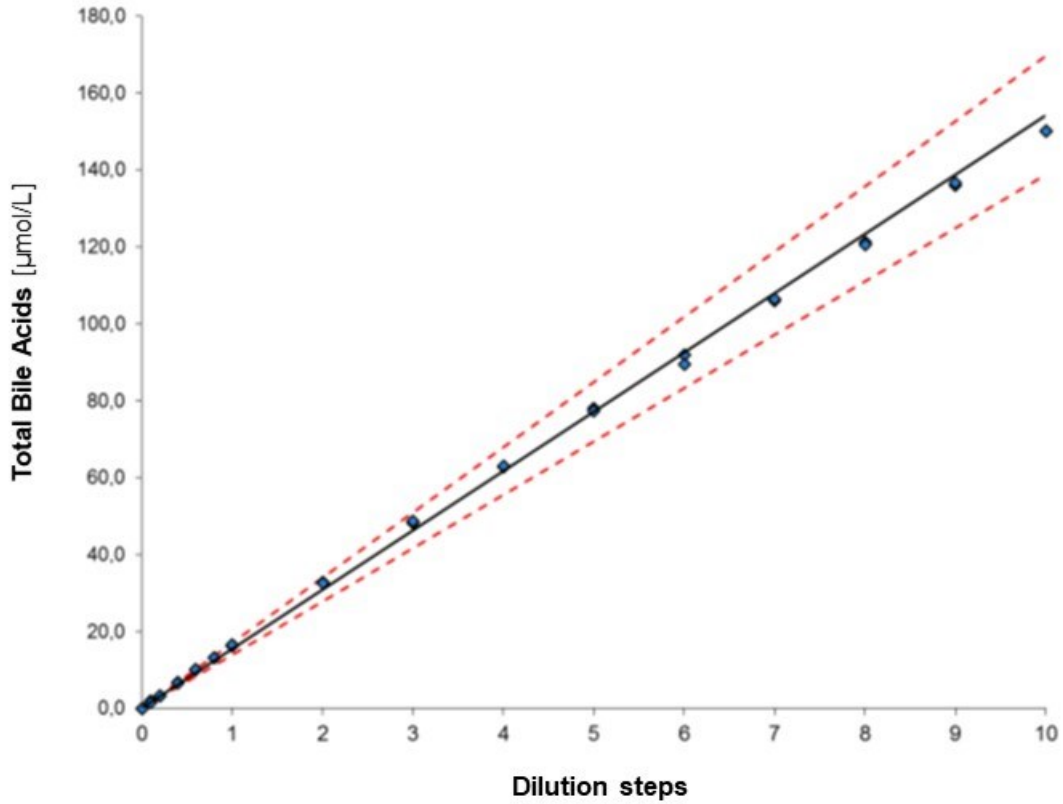
Supplementary Table S4. Within-run precision (repeatability; n=20) of DiaSys TBA 21 FS on three different clinical chemistry analyzers.

Analyzer		Sample 1 (stool)	Sample 2 (stool)
respons® 910 Benchtop	Mean [µmol/L]	17.8	140
	CV [%]	**2.22**	**1.98**
BioMajesty® JCA-BM6010/C Automated random access	Mean [µmol/L]	17.5	144
	CV [%]	**1.04**	**0.60**
respons® 940 Automated random access	Mean [µmol/L]	17.4	142
	CV [%]	**1.41**	**0.58**

Supplementary Figure S1. Linearity assessment for TBA 21 FS run on respons® 910. The linear range of the Total bile acids 21 FS assay (DiaSys) was assessed according to CLSI acceptance criteria (CLSI EP05, 3rd Edition) by serial dilution of two different stool extracts for **(A)** lower linearity (0-50 µmol/L; ±15% deviation) and **(B)** upper linearity (0-170 µmol/L; ±10% deviation).



Supplementary Figure S2. Linearity assessment for TBA 21 FS run on respons® 940. A stool extract was spiked to a final concentration of 150 µmol/L, serially diluted with IDK Extract® buffer, and each dilution was measured in duplicate (red dotted line: ±10% deviation).



Supplementary Figure S3. Comparison of DiaSys TBA 21 FS run on different clinical chemistry analyzers. **(A)** respons® 940 vs. respons® 910 and **(B)** respons® 940 vs. BioMajesty® JCA-BM6010/C. Stool samples (n=20) were processed using a ready-to-use stool sampling device (IDK Extract® specimen tubes, Immundiagnostik AG, Bensheim, Germany) according to the manufacturer's instructions. The resulting stool extracts were measured on the indicated analyzers.