

SUPPORTING INFORMATION

TITLE: Inter-laboratory reproducibility of a targeted metabolomics platform for analysis of human serum and plasma

Alexandros P. Siskos,^{1§} Pooja Jain,^{1§} Werner Römisch-Margl,² Mark Bennett,³ David Achaintre,⁴ Yasmin Asad,⁵ Luke Marney,⁶ Larissa Richardson,⁶ Albert Koulman,⁶ Julian L. Griffin,⁶ Florence Raynaud,⁵ Augustin Scalbert,⁴ Jerzy Adamski,^{7,8,9} Cornelia Prehn,⁷ Hector C. Keun^{1*}

¹Department of Surgery and Cancer, Imperial College London, W12 0NN, UK,

²Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München, German Research Center for Environmental Health, 85764 Neuherberg, Germany

³Department of Life Sciences, Imperial College London, SW7 2AZ, UK

⁴International Agency for Research on Cancer (IARC), Biomarkers Group, F-69372 Lyon, France,

⁵The Institute of Cancer Research, ICR, Sutton, SM2 5NG, UK,

⁶MRC Human Nutrition Research, Cambridge, CB1 9NL, UK,

⁷Genome Analysis Center, Institute of Experimental Genetics, Helmholtz Zentrum München, German Research Center for Environmental Health, 85764 Neuherberg, Germany

⁸Chair of Experimental Genetics, Center of Life and Food Sciences Weihenstephan, Technische Universität München, 85354 Freising-Weihenstephan, Germany

⁹German Center for Diabetes Research (DZD), 85764 Neuherberg, Germany

[§]These authors contributed equally

*Corresponding author. Hector C. Keun, e-mail: h.keun@imperial.ac.uk

Summary of Supporting Information

Supplementary Figure S-1. Run order and 96-well plate layout, with vertical pipetting and run order mode.

Supplementary Figure S-2. Outlier identification with PCA analysis.

Supplementary Figure S-3. Laboratory batch effects depicted with PCA.

Supplementary Table S-1. Participating population, material and test samples.

Supplementary Table S-2. List of missing and excluded data.

Supplementary Table S-3. Assay Performance (accuracy and reproducibility) of spiked QC samples at three concentrations for 59 metabolites (42 LC and 17 FIA).

Supplementary Table S-4. Effect of normalisation to inter-laboratory %CV.

Supplementary Table S-5. List of the 189 metabolites detected with median intra-laboratory %CV, % BLD and % missing values per laboratory, also median inter-laboratory %CV (prior and post normalisation), and total % BLD and % missing values, for the 20 test material.

Supplementary Table S-6. Median intra-laboratory %CV, median inter-laboratory %CV (prior and post normalisation), and per laboratory % BLD and % missing values, for the 20 test material and 189 metabolites, per metabolite class.

Supplementary Table S-7. Ratio of inter-laboratory to intra-laboratory % CV, for the 20 test materials and 189 metabolites, per metabolite class for the six participating laboratories.

Supplementary Table S-8. List of metabolites with BLD + missing >30% and/or median inter-lab %CV >20%. BLD: below the limit of detection.

Supplementary Table S-9. Medians of inter-laboratory %CV for the 20 test material, the serum samples and plasma samples with different anticoagulants, the high lipid content samples and the pooled QC (QCP).

Supplementary Table S-10. Estimation of overall and per laboratory accuracy and precision with reference to NIST SRM 1950.

Supplementary Table S-11. Inter-batch precision %CV (plate to plate variability) for laboratory A for two citrate plasma QCs, 2 replicates each per plate, for 14 plates.

Supplementary Table S-12. Inter-batch precision %CV (plate to plate variability) for laboratory B for two QC materials, the SRM NIST 1950 and a commercial available human serum (CQC). Also mean intra-batch precision %CV for the SRM NIST 1950. 4 replicates each per plate, for 4 plates for the SRM NIST 1950 and 2 replicates each per plate, for 4 plates for CQC.

Supplementary Table S-13. Inter-batch precision %CV (plate to plate variability) and mean intra-batch precision %CV for lab C, for a commercially available reference plasma, 5 replicates each per plate, for 4 plates.

Supplementary Table S-14. Baseline analytical performance of the kit and performance limitations in normal human plasma, as provided by the kit manufacturer. CV calculated with 6 replicates of QC samples per plate, for 4 plates.

Supplementary Table S-15. Limits of detection (LODs) for each metabolite calculated by each of the participating laboratory.

Supplementary Table S-16. Concentrations of standards used for calibration curves (μM).

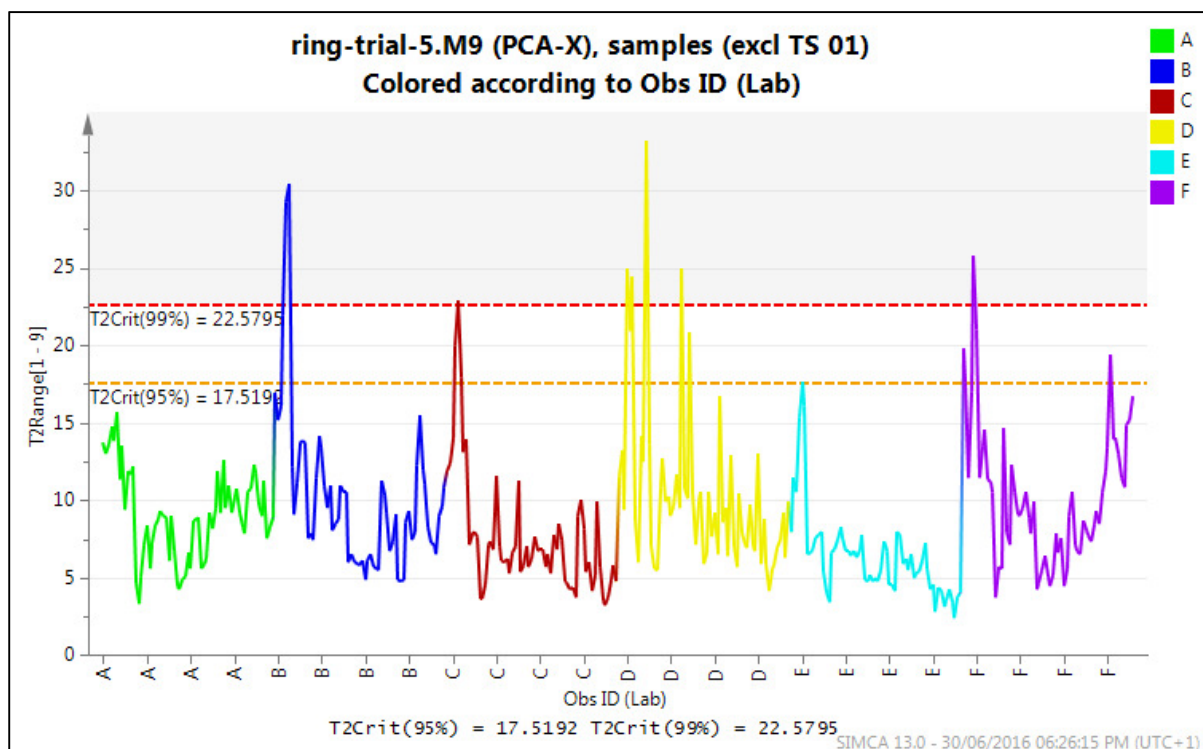
Supplementary Table S-17. Intra-lab CVs calculated with the requirement of minimum $n=3$ and $n=2$ for the 20 test material.

Supplementary Protocol S-1. Basic common guidance and checklist for instrument cleaning and benchmarking prior to analysis.

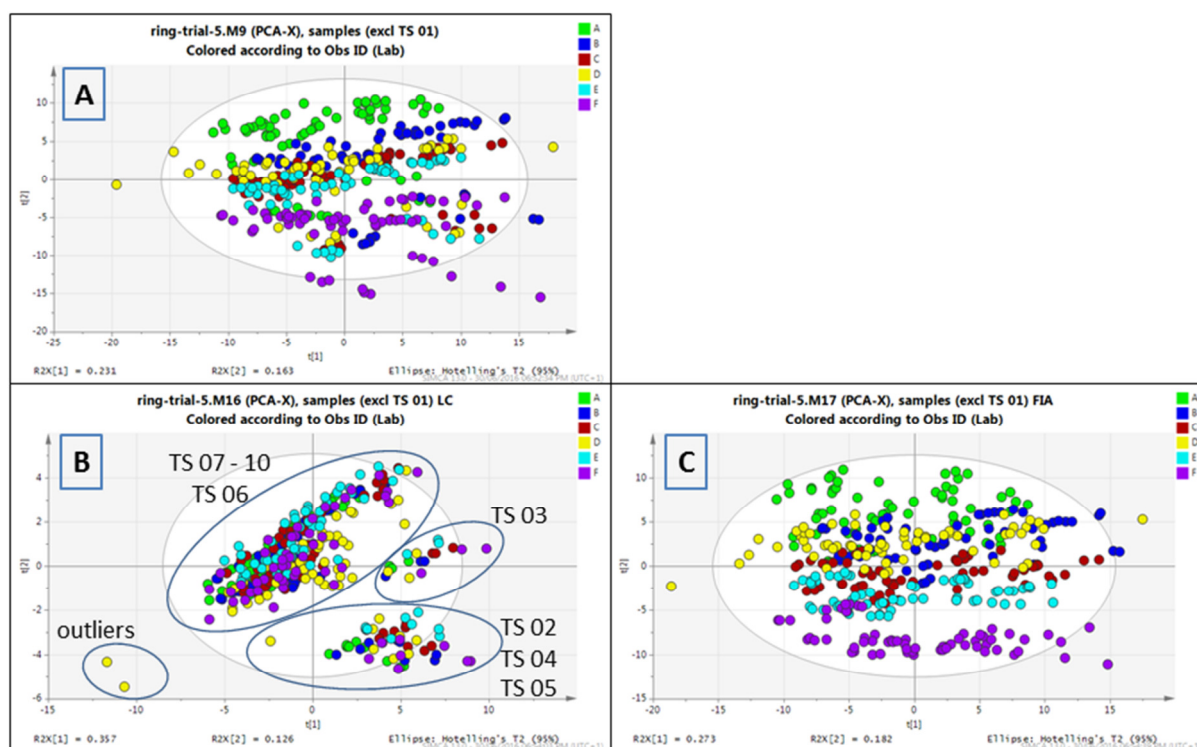
Supplementary Protocol S-2. Metabolomics measurements - Laboratory instrumentation.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank 1	Cal 5 2	Kit - QC 2 3	TS 02 4	TS 04 5	TS 07-1 6	TS 07-3 7	TS 08-2 8	TS 08-4 9	TS 09-3 10	TS 10-1 11	TS 10-4 12
B	Zero (PBS) 13	Cal 6 14	Kit - QC 3 15	TS 02 16	TS 05 17	TS 07-1 18	TS 07-4 19	TS 08-2 20	QC Pool 21	TS 09-3 22	TS 10-1 23	TS 10-4 24
C	Zero (PBS) 25	Cal 7 26	Kit - QC 3 27	TS 03 28	TS 05 29	TS 07-1 30	TS 07-4 31	TS 08-2 32	TS 09-1 33	TS 09-3 34	TS 10-2 35	TS 10-4 36
D	Zero (PBS) 37	Kit - QC 1 38	Kit - QC 3 39	TS 03 40	TS 05 41	TS 07-2 42	TS 07-4 43	TS 08-3 44	TS 09-1 45	TS 09-4 46	TS 10-2 47	Kit - QC 1 48
E	Cal 1 49	Kit - QC 1 50	TS 01 51	TS 03 52	TS 06 53	TS 07-2 54	QC Pool 55	TS 08-3 56	TS 09-1 57	TS 09-4 58	TS 10-2 59	Kit - QC 1 60
F	Cal 2 61	Kit - QC 1 62	TS 01 63	QC Pool 64	TS 06 65	TS 07-2 66	TS 08-1 67	TS 08-3 68	TS 09-2 69	TS 09-4 70	TS 10-3 71	Kit - QC 2 72
G	Cal 3 73	Kit - QC 2 74	TS 01 75	TS 04 76	TS 06 77	TS 07-3 78	TS 08-1 79	TS 08-4 80	TS 09-2 81	QC Pool 82	TS 10-3 83	Kit - QC 2 84
H	Cal 4 85	Kit - QC 2 86	TS 02 87	TS 04 88	QC Pool 89	TS 07-3 90	TS 08-1 91	TS 08-4 92	TS 09-2 93	TS 10-1 94	TS 10-3 95	QC Pool 96

Supplementary Figure S-1: Run order and 96-well plate layout, with vertical pipetting and run order mode.



Supplementary Figure S-2. Outlier identification with PCA analysis concluded in two outliers TS05-D-17 and TS07-3-D-90. Depicted a PCA model (9 components), of all test material (excluded TS01). Samples with $T2Range[1-9] > 99\%$ were identified as possible outliers. These were 8 samples: TS07-3-D-90, TS05-D-17, TS03-B-40, TS03-B-52, TS03-C-40, TS03-D-40, TS03-D-52, TS03-F-40. After further investigation, it was established that the TS03 samples were not outliers, but this was due to real biological variation, compared to the rest of the test materials. Finally two outliers were confirmed and removed from subsequent analysis (Lab D). For these outliers it was confirmed that the values of most of the reported metabolites are 30-50% of the values of the other replicates of same test material.



Supplementary Figure S-3. Laboratory batch effects depicted with PCA. Initial PCA indicated that systematic differences were present between the laboratories in the metabolic profile. The overall batch effects could be attributed to the FIA metabolites. Figure S3 depicts the PCA analysis of all the test samples TS 02 to 06 and TS 07-1 to 10-4 (high lipid TS 01 was excluded). (A): all metabolites, (B): LC metabolites and (C): FIA metabolites.

Supplementary Table S-1: Participating population, material and test samples.

Sample name	Description	Origin	Repl.
Blank	No sample, no IS		1
Zero (PBS)	PBS sample (phosphate-buffered saline)		3
cal1-7	Calibration Standards, 7 concentrations	provided with the kit	7 x 1
p180-QC1	Kit-QC p180-MetaDis QC level 1	provided with the kit barcode # 438006	5
p180-QC2	Kit-QC p180-MetaDis QC level 2	provided with the kit barcode # 438010	5
p180-QC3	Kit-QC p180-MetaDis QC level 3	provided with the kit barcode # 438023	3
TS 06	NIST SRM 1950, lithium heparin plasma	NIST	3
TS 01	Individual 1, EDTA plasma, male 32 years, high lipid	in.vent DIAGNOSTICA GMBH, Lot# 200898	3
TS 02	Individual 2, EDTA plasma, female 25 years, healthy	in.vent DIAGNOSTICA GMBH, Lot# 222360	3
TS 03	Individual 3, EDTA plasma, female 54 years, healthy	in.vent DIAGNOSTICA GMBH, Lot# 221668	3
TS 04	Individual 4, EDTA plasma, male 59 years, healthy	in.vent DIAGNOSTICA GMBH, Lot# 212046	3
TS 05	Individual 5, EDTA plasma, male 27 years, healthy	in.vent DIAGNOSTICA GMBH, Lot# 211343	3
TS 07-1	Individual 7, serum, healthy volunteer,	Royal Marsden Hospital, London	3
TS 07-2	Individual 7, EDTA plasma		3
TS 07-3	Individual 7, citrate plasma		3
TS 07-4	Individual 7, heparin plasma		3
TS 08-1	Individual 8, serum, healthy volunteer,	Royal Marsden Hospital, London	3
TS 08-2	Individual 8, EDTA plasma		3
TS 08-3	Individual 8, citrate plasma		3
TS 08-4	Individual 8, heparin plasma		3
TS 09-1	Individual 9, serum, healthy volunteer,	Royal Marsden Hospital, London	3
TS 09-2	Individual 9, EDTA plasma		3
TS 09-3	Individual 9, citrate plasma		3
TS 09-4	Individual 9, heparin plasma		3
TS 10-1	Individual 10, serum, healthy volunteer,	Royal Marsden Hospital, London	3
TS 10-2	Individual 10, EDTA plasma		3
TS 10-3	Individual 10, citrate plasma		3
TS 10-4	Individual 10, heparin plasma		3
QC pool	pool of all Test Samples 07-1 to 10-4	Prepared in each participating laboratory	6

TS: test sample, Repl: number of replicates, IS: internal standards

Supplementary Table S-2. List of missing and excluded data.

Metabolite	Samples	Laboratories	Comment
Total DMA	All	Lab D and Lab F (UHPLC used)	Data not acquired, NQ
Sarcosine	All	Lab A, B, C and E (LC used)	Data not acquired, NQ
PC aa C30:2	All	Lab F (Waters instrument)	Data not acquired, NQ
PC aa C32:2	All	Lab F (Waters instrument)	Data not acquired, NQ
PC aa C38:1	All	Lab F (Waters instrument)	Data not acquired, NQ
SM C22:3	All	Lab F (Waters instrument)	Data not acquired, NQ
All	All	All	'NA'
All	All	All	Zero, '0'
All	TS05-D-17 TS07-3-D-90	Lab D	Outliers in PCA scores (Figure S-2)
Alanine, Asparagine, Aspartate, Citrulline, Glutamate, Threonine, ADMA, SDMA, total DMA, Creatinine, DOPA, Dopamine, PEA Putrescine, Sarcosine, Serotonin, Spermidine, Spermine, Taurine	TS07-1-F-6 TS07-1-F-18 TS10-1-F-11 TS10-1-F-23	Lab F	Experimental error – Internal standards not added
Histamine	TS02-A-4	Lab A	Outlier – visual inspection of raw data
All	QC pool-D-21 QC pool-D-64 QC pool-D-96 QC pool-D-55 QC pool-D-82 QC pool-D-89	Lab D	Experimental error in sample preparation (personal communication from laboratory)

BS3-F-40: the value for ADMA was originally reported as zero. This was recalculated by the lab, replaced with 0.4891 μM .

Supplementary Table S-3. Assay Performance (accuracy and reproducibility) of spiked QC samples at three concentrations for 59 metabolites (42 LC and 17 FIA) for which nominal levels were provided by the manufacturer (n=5 for low and medium level, n=3 for high level). Data provided for the overall performance and also per laboratory (NA: calculation not possible due to missing data, NV: no nominal value provided, NQ: data not acquired, for sarcosine data was acquired only for 2 laboratories). For 8 metabolites (spermine, spermidine, t-4-OH-Pro, carnosine, nitro-Tyr, Glu, c-4-OH-Pro, and PEA), the poor inter-laboratory reproducibility, could be attributed to the poor accuracy of 1 outlying laboratory (see text). Excluding data from the outlying laboratory for these 8 metabolites brought inter-laboratory reproducibility to within 20%:

Glu:	%CV QC1 = 4.8,	%CV QC2 = 6.7,	%CV QC3 = 6.1
Spermidine:	%CV QC1 = 14.2,	%CV QC2 = 7.7,	%CV QC3 = 7.6.
Spermine:	%CV QC1 = 12.6,	%CV QC2 = 8.1,	%CV QC3 = 6.8.
t-4-OH-Pro:	%CV QC1 = 2.8,	%CV QC2 = 8.7,	%CV QC3 = 8.1.
c4-OH-Pro:	%CV QC1 = 6.5,	%CV QC2 = 7.8,	%CV QC3 = 8.9.
Carnosine:	%CV QC1 = 9.6,	%CV QC2 = 10.2,	%CV QC3 = 12.8.
Nitro-Tyr:	%CV QC1 = 11.4,	%CV QC2 = 7.4,	%CV QC3 = 14.7.
PEA:	%CV QC1 = NV	%CV QC2 = 7.4,	%CV QC3 = 7.1.

Supplementary Table S-3

Metabolite	Nominal values (µM)			Lab A									Lab B									Lab C									Lab D									Lab E									Lab F									ALL								
				Accuracy %			reproducibility %			Accuracy %			reproducibility %			Accuracy %			reproducibility %			Accuracy %			reproducibility %			Accuracy %			reproducibility %			Accuracy %			reproducibility %			Accuracy %			reproducibility %			Accuracy %			reproducibility %																	
FIA-MS/MS	QC1	QC2	QC3	QC1	QC2	QC3	QC1	QC2	QC3	QC1	QC2	QC3	QC1	QC2	QC3	QC1	QC2	QC3	QC1	QC2	QC3	QC1	QC2	QC3	QC1	QC2	QC3	QC1	QC2	QC3	QC1	QC2	QC3	QC1	QC2	QC3	QC1	QC2	QC3	QC1	QC2	QC3	QC1	QC2	QC3	QC1	QC2	QC3																		
C0	30	52	75	109.1	106.3	106.6	3.0	3.3	1.0	96.6	93.3	101.6	4.4	6.3	3.5	104.0	106.6	99.7	5.4	11.5	21.0	118.1	120.9	119.3	10.0	13.0	5.3	88.2	91.9	91.3	7.2	6.1	2.2	94.6	97.0	94.4	5.2	4.3	6.5	101.8	102.7	102.1	10.7	10.6	9.8																					
C10	0.49	1.8	3.4	125.4	112.7	111.5	8.4	4.2	2.2	104.4	100.4	114.4	4.0	9.2	3.6	120.5	127.6	115.4	4.5	12.4	17.5	91.4	94.5	80.5	13.7	17.0	7.0	100.3	103.2	112.1	10.0	7.4	3.4	96.1	106.9	119.0	6.2	10.7	7.5	106.4	107.6	108.8	12.8	10.7	13.0																					
C12	0.72	3.9	7.7	96.3	96.4	93.9	7.6	3.2	2.3	99.6	98.5	112.8	5.4	9.1	3.3	150.5	160.4	142.6	4.7	9.4	14.4	83.5	97.4	81.7	12.6	16.2	8.4	102.7	106.6	115.9	8.7	5.5	0.2	96.8	97.5	107.1	8.5	9.8	19.8	104.9	109.5	109.0	22.2	23.1	19.1																					
C14	0.62	1.7	3.3	123.7	116.8	122.1	5.5	3.4	2.4	101.7	97.3	109.1	6.7	7.2	2.4	118.8	126.0	115.3	3.5	10.2	19.7	114.9	104.2	104.9	8.7	12.8	8.7	102.1	103.2	114.7	7.5	3.9	1.6	90.1	84.8	95.3	6.4	10.5	10.4	108.6	105.4	110.2	11.7	13.7	8.5																					
C16	0.64	3.6	7	116.7	109.4	114.2	4.7	3.9	0.9	104.0	98.6	107.5	3.1	8.3	2.8	122.1	125.3	113.1	5.5	10.7	16.3	112.6	112.1	114.0	6.2	12.2	10.2	109.5	109.1	119.0	8.4	5.3	0.4	111.6	104.3	111.5	7.0	5.3	12.6	115.6	109.7	113.2	8.0	8.3	3.3																					
C18	0.72	1.9	3.8	104.8	104.7	105.0	4.7	5.0	1.7	100.1	100.4	108.9	4.1	7.3	5.5	111.7	120.5	103.0	3.6	10.6	17.2	119.6	114.5	118.2	9.9	13.6	11.7	99.7	104.3	111.2	8.9	8.0	2.0	98.6	95.3	97.5	6.7	6.4	11.0	105.7	106.6	107.3	7.9	8.7	6.7																					
C2		4	12	21	102.5	100.7	101.8	3.1	3.1	1.4	98.0	96.8	103.8	3.1	8.9	5.0	112.1	113.0	96.1	4.3	10.9	17.5	114.4	109.9	96.5	6.6	14.5	9.5	100.1	99.5	107.5	6.7	6.8	2.3	93.6	93.1	87.8	7.0	5.9	7.4	103.4	102.2	98.9	7.9	7.5	7.1																				
C3	0.62	4.6	8.8	113.4	104.3	111.5	2.9	4.2	0.9	107.1	100.2	112.2	7.6	9.3	1.6	123.2	118.5	108.2	4.5	10.9	16.7	121.9	95.9	94.9	5.9	10.0	8.4	111.8	108.0	117.3	2.9	7.9	2.8	74.3	68.6	70.4	5.3	6.8	4.7	108.6	99.2	102.4	16.5	17.0	17.0																					
C4	0.71	4.1	8	102.8	97.6	101.3	4.1	1.9	1.7	99.9	94.6	104.6	4.6	7.5	2.6	121.9	119.2	108.8	3.3	12.0	17.7	103.6	103.0	98.6	6.8	11.2	8.4	94.7	93.3	101.5	5.1	5.7	4.8	94.8	90.7	91.8	6.8	10.0	2.6	102.8	99.7	101.1	9.8	10.5	5.7																					
C5	0.79	4.6	9.1	94.9	87.9	93.3	2.6	4.5	2.4	85.5	79.6	86.1	4.8	8.3	4.4	113.3	108.3	98.7	5.1	12.6	15.7	101.7	91.4	90.3	8.2	10.4	8.4	95.5	97.7	98.5	8.0	7.1	1.7	80.4	76.5	76.7	5.2	9.9	7.2	95.2	90.3	90.6	12.3	13.0	9.2																					
C6 (C4:1-DC)	0.38	2.2	4.4	119.4	85.9	85.2	17.4	4.5	2.3	77.1	75.3	84.4	2.3	9.0	1.3	108.5	109.2	93.3	2.7	12.4	12.2	109.6	92.4	96.0	10.0	11.2	11.3	88.7	88.3	95.4	10.9	6.7	5.4	80.1	78.0	81.4	2.9	6.8	5.8	97.2	88.2	89.3	18.1	13.7	7.1																					
C8	0.3	2.1	4.2	164.5	128.7	127.0	5.6	4.4	1.9	112.0	96.2	99.2	5.5	11.4	2.4	129.8	137.0	124.0	3.1	12.2	18.6	149.4	135.7	130.0	8.6	12.7	6.4	129.1	116.9	123.7	15.3	5.7	0.5	96.2	97.5	97.2	6.3	5.6	12.7	130.2	118.7	116.8	18.9	15.5	12.5																					
lysOPC a C18:0	40	84	127	126.7	121.6	127.6	13.6	15.5	2.1	118.5	114.7	121.4	5.5	7.6	3.5	108.1	117.7	99.0	5.4	9.2	19.6	151.4	128.9	123.2	10.0	12.7	11.4	93.0	91.7	98.3	6.5	8.1	2.0	131.1	126.1	109.9	23.0	21.0	17.3	121.5	115.8	113.2	16.5	11.6	11.2																					
PC aa C24:0	6.8	21	41	103.5	98.7	101.2	2.6	5.3	0.5	116.2	111.7	126.6	4.7	8.2	5.0	116.3	114.5	106.1	3.6	10.4	16.3	123.2	112.3	117.0	8.5	13.1	9.3	94.9	95.2	102.5	5.6	6.1	2.1	88.8	83.2	88.3	5.1	4.7	10.0	107.1	102.6	107.0	12.7	12.1	12.4																					
PC aa C34:1	118	118	118	118.6	116.4	112.7	10.0	7.3	1.9	128.5	129.0	129.8	5.6	7.1	3.5	106.7	104.0	100.2	3.8	11.3	18.1	117.5	114.6	115.4	8.1	11.1	10.2	94.8	98.9	105.5	5.4	5.8	4.5	88.4	87.6	81.6	19.4	18.6	11.1	109.6	109.5	107.5	13.5	13.2	15.1																					
SM C18:0	21	21	21	73.2	72.4	72.7	7.4	9.7	4.5	50.5	48.6	49.5	5.6	6.2	3.2	102.0	104.4	94.8	3.5	9.2	18.0	93.3	91.2	90.2	9.3	11.4	13.4	94.5	94.0	103.6	6.8	8.1	3.4	197.2	191.7	165.5	25.2	25.6	14.8	101.8	100.4	96.9	49.5	48.7	39.7																					
H1	6731	13236	19453	84.6	96.4	101.0	3.0	3.2	0.4	85.4	85.1	95.9	3.6	8.0	2.2	110.9	112.3	102.6	3.4	11.3	17.6	92.4	102.8	104.7	7.8	12.6	9.2	114.5	112.5	120.6	7.1	5.6	1.6	106.6	107.8	91.0	15.0	22.6	8.5	99.1	102.8	102.6	13.4	10.3	9.9																					
LC-MS/MS																																																																		
Ala	336	758	1209	95.5	97.0	91.2	3.1	3.7	3.1	94.4	94.4	93.7	1.0	2.5	0.8	97.3	104.4	85.6	4.6	6.8	20.1	105.9	100.7	93.5	7.1	4.6	7.0	98.5	97.5	91.0	3.6	1.6	3.3	97.2	102.4	95.7	4.6	3.3	7.4	98.1	99.4	91.8	4.1	3.8	3.8																					
Arg	93	190	282	86.9	91.9	93.9	6.0	6.7	6.1	96.5	90.7	104.1	2.5	8.2	2.0	93.2	93.7	81.0	6.1	13.7	14.4	115.7	109.7	110.8	6.1	11.3	11.6	87.4	84.5	90.1	4.2	10.0	5.4	93.6	98.1	95.9	6.9	6.8	8.5	95.2	94.8	96.0	11.1	9.0	10.9																					
Asn	21.1	144	270	97.4	92.1	88.9	3.9	4.0	5.4	94.4	104.0	112.7	1.5	4.9	2.7	107.9	109.9	90.9	4.5	10.1	18.0	87.5	111.1	101.2	11.3	9.0	8.6	104.5	114.0	105.9	10.5	7.4	6.4	103.6	112.1	98.5	6.0	3.4	4.3	99.5	107.2	99.7	7.6	7.6	9.0																					
Asp	10	141	283	91.4	90.4	93.4	9.1	2.4	6.2	98.3	104.0	103.4	12.6	6.5	2.1	85.4	115.5	88.1	13.5	8.5	13.8	124.2	117.7	99.4	31.5	8.2	6.4	121.0	115.5	112.0	5.9	11.6	3.6	103.7	111.0	98.9	21.0	8.4	6.5	104.0	109.1	99.2	15.1	9.4	8.3																					
Cit	25	156	291	91.4	89.4	89.6	4.5	4.3	4.7	99.0	100.8	98.1	3.1	3.4	3.0	102.6	106.4	88.9	7.5	10.8	20.1	125.4	107.2	96.6	4.5	7.9	12.0	113.1	114.6	109.9	10.1	13.4	10.3	99.8	104.7	95.7	5.9	6.2	2.1	105.2	103.9	96.5	11.5	8.1	7.8																					
Gln	409	823	1253	82.4	78.8	83.8	3.5	6.0	1.1	86.4	83.2	90.2	4.3	6.3	0.9	93.7	90.3	75.3	6.3	7.5	15.3	70.3	80.8	79.8	9.4	14.2	11.3	99.2	94.2	98.4	12.1	12.2	6.2	87.0	84.9	84.4	6.4	5.4	9.0	86.5	85.4	85.4	11.5	6.8	9.5																					
Glu	124	353	600	92.3	97.0	91.6	4.1	4.6	1.7	89.7	95.7	93.1	2.0	2.2	2.8	96.8	108.9	88.2	8.8	5.5	19.2	151.6	117.3	109.6	7.5	13.2	13.9	86.1	110.5	104.5	23.3	8.1	6.0	97.4	101.8	94.4	7.2	5.5	2.5	102.3	105.2	96.9	24.0	8.0	8.6																					
Gly	190	845	1471	83.4	75.0	78.0	5.1	10.3	4.8	93.0	91.7	103.5	3.5	9.1	3.8	109.2	102.4	85.4	7.3	12.7	13.5	111.4	95.2	96.8	7.2	11.0	10.8	101.1	87.1	89.3	13.0	8.5	0.4	92.0	90.6	93.5	4.5	5.4	8.0	98.3	90.3	91.1	11.0	10.1	9.8																					
His	70	191	313	85.9	80.7	81.9	2.2	7.3	1.1	89.8	85.3	93.7	3.2	8.6	1.4	95.7	88.4	73.3	6.4	8.7	10.7	101.1	96.2	95.2	7.9	12.4	9.7	96.5	88.1	88.5	6.4	8.7	4.3	90.9	88.6	86.0	4.5	4.5	4.2	93.3	87.9	86.4	5.8	5.8	9.4																					
Ile	79	189	300	90.0	88.4	89.2	4.4	5.7	1.6	90.6	88.5	97.1	3.6	8.0	2.8	107.4	107.6	93.0	7.2	8.8	14.1	95.3	106.0	113.2	8.4	13.7	10.6	113.5	110.8	113.9	9.4	6.6	2.8	91.8	90.8	89.7	6.3	6.5	7.6	98.1	98.7	99.4	10.1	10.7	11.4																					
Leu	138	215	292	87.1	86.5	87.8	5.7	5.2	0.3	93.6	93.3	101.2	2.2	8.8	3.4	105.2	108.9	93.9	7.0	7.4	14.2	106.5	111.7	116.4	9.3	15.0	11.5	114.1	115.0	128.4	11.2	7.4	7.4	90.7	93.3	94.9	9.2	4.5	7.7	99.5	102.1	103.8	10.6	11.1	15.0																					
Lys	191	408	622	70.0	73.4	76.0	1.8	11.2	5.3</																																																									

Supplementary Table S-4. Effect of normalisation to inter-laboratory %CV for the 20 test material and 189 metabolites (total 3780 inter-lab %CV values), depicted per metabolite class. Data is shown for the non-normalised data, the SRM 1950 normalised (mean and median) and the spiked QC level 2 normalised (mean and median).

Supplementary Table S-4

	Non Normalised					SRM 1950 Mean Normalised					SRM 1950 Median Normalised					QC2 Mean Normalised						QC2 Median Normalised				
LC-MS/MS	< 10%	10-20%	20-30%	> 30%	Excluded	< 10%	10-20%	20-30%	> 30%	Excluded	< 10%	10-20%	20-30%	> 30%	Excluded	< 10%	10-20%	20-30%	> 30%	Excluded		< 10%	10-20%	20-30%	> 30%	Excluded
Amino Acids	298	86	4	32	0	346	36	6	32	0	335	46	5	34	0	324	60	8	28	0		333	52	7	28	0
Biogenic Amines	74	47	44	147	128	110	59	39	90	142	111	50	42	95	142	39	53	103	116	129		38	56	98	119	129
FIA-MS/MS																										
Carnitines	81	195	104	420	0	312	370	88	30	0	265	386	117	32	0	148	369	153	130	0		146	360	168	126	0
Di-acyl PC (PC aa)	72	436	144	108	0	602	107	24	27	0	579	130	24	27	0	395	249	61	55	0		341	292	69	58	0
Alkyl-acyl PC (PC ae)	85	507	106	62	0	629	92	13	26	0	595	125	14	26	0	338	217	101	104	0		339	213	105	103	0
Lyso PC	21	131	30	98	0	145	78	33	24	0	135	92	34	19	0	154	88	29	9	0		125	99	43	13	0
Sphingolipids	0	0	12	270	18	207	65	6	4	18	191	79	8	3	19	213	59	6	4	18		178	93	7	4	18
Sum of hexoses	12	7	1	0	0	14	5	1	0	0	14	5	1	0	0	15	5	0	0	0		14	6	0	0	0
Total	643	1409	445	1137	146	2365	812	210	233	160	2225	913	245	236	161	1626	1100	461	446	147		1514	1171	497	451	147

Supplementary Table S-5. List of the 189 metabolites detected with median intra-laboratory %CV, % BLD and % missing values per laboratory, also median inter-laboratory %CV (prior and post normalisation), and total % BLD and % missing values, for the 20 test material. The column indicating missing includes the total of not acquired data 'NQ', 'NA' values, zero values and outliers. Highlighted values for median inter-laboratory %CV >20% and BLD+missing values >30 %.

Supplementary Table S-5

Analyte	LAB A			LAB B			LAB C			LAB D			LAB E			LAB F			Measure- used n	All labs				
	median CV	%<LOD	%missing	median CV	%<LOD	%missing	median CV	%<LOD	%missing	median CV	%<LOD	%missing	median CV	%<LOD	%missing	median CV	%<LOD	%missing		Median InterLab %CV [90%CI] Non-Normalised	Median InterLab %CV [90%CI] Normalised	%<LOD	%missing	% total (BLD +missing)
C0	4.0	0.0	0.0	5.0	0.0	0.0	4.1	0.0	0.0	6.9	0.0	3.3	5.1	0.0	0.0	7.1	0.0	0.0	358	8.1 [6.4-11]	5 [2.9-6.3]	0.0	0.6	0.6
C10	5.0	43.3	0.0	5.1	0.0	0.0	4.7	0.0	0.0	5.8	11.7	3.3	5.7	0.0	0.0	8.4	0.0	0.0	358	19 [15-24]	9.1 [5.1-14]	9.2	0.6	9.7
C10:1	7.1	100.0	0.0	6.3	60.0	0.0	5.9	0.0	0.0	8.3	50.0	3.3	10.9	100.0	0.0	11.8	98.3	0.0	358	22 [18-25]	6.3 [3.5-9.6]	68.1	0.6	68.6
C10:2	7.6	100.0	0.0	5.7	100.0	0.0	7.3	38.3	0.0	9.3	96.7	3.3	9.4	70.0	0.0	6.1	40.0	0.0	358	20 [17-22]	9.3 [6.5-16]	74.2	0.6	74.7
C12	5.9	15.0	0.0	5.8	8.3	0.0	6.6	0.0	0.0	7.5	53.3	3.3	6.5	1.7	0.0	7.2	1.7	0.0	358	13 [12-16]	8.4 [6.4-10]	13.3	0.6	13.9
C12-DC	6.6	100.0	0.0	5.3	100.0	0.0	3.7	100.0	0.0	6.2	96.7	3.3	5.3	100.0	0.0	5.1	100.0	0.0	358	60 [56-66]	13 [7.6-20]	99.5	0.6	100.0
C12:1	7.6	100.0	0.0	6.2	30.0	0.0	7.0	6.7	0.0	8.7	80.0	3.3	9.9	100.0	0.0	9.2	100.0	0.0	358	18 [15-22]	9.8 [4.9-12]	69.5	0.6	70.0
C14	6.1	100.0	0.0	7.4	51.7	0.0	4.2	0.0	0.0	7.8	96.7	3.3	7.5	40.0	0.0	5.8	11.7	0.0	358	44 [31-54]	18 [11-25]	50.0	0.6	50.6
C14:1	5.1	3.3	0.0	6.6	0.0	0.0	5.5	0.0	0.0	7.2	10.0	3.3	5.3	0.0	0.0	7.6	0.0	0.0	358	18 [14-26]	12 [7.9-17]	2.2	0.6	2.8
C14:1-OH	7.4	100.0	0.0	7.6	78.3	0.0	8.6	13.3	0.0	8.8	96.7	3.3	10.0	28.3	0.0	7.9	0.0	0.0	358	52 [44-61]	16 [7.4-24]	52.8	0.6	53.3
C14:2	7.5	23.3	0.0	7.9	1.7	0.0	6.2	0.0	0.0	10.4	80.0	3.3	8.0	1.7	0.0	7.3	0.0	0.0	358	17 [14-21]	7.9 [4.5-13]	17.8	0.6	18.3
C14:2-OH	7.3	100.0	0.0	8.9	98.3	0.0	12.4	98.3	0.0	11.6	96.7	3.3	12.7	73.3	0.0	10.4	0.0	0.0	358	56 [46-62]	9.8 [6-16]	77.8	0.6	78.3
C16	4.6	0.0	0.0	5.1	0.0	0.0	5.8	0.0	0.0	6.5	0.0	3.3	5.3	0.0	0.0	6.5	0.0	0.0	358	8.3 [4.9-12]	7.3 [5.9-9.3]	0.0	0.6	0.6
C16-OH	8.5	100.0	0.0	11.5	100.0	0.0	9.1	100.0	0.0	8.3	95.0	3.3	14.2	100.0	0.0	8.8	0.0	0.0	358	68 [59-75]	12 [9.6-21]	82.5	0.6	83.1
C16:1	6.4	100.0	0.0	7.5	85.0	0.0	5.3	43.3	0.0	7.4	96.7	3.3	4.5	70.0	0.0	8.1	5.0	0.0	358	23 [16-30]	8.1 [5.3-13]	66.7	0.6	67.2
C16:1-OH	8.6	100.0	0.0	7.9	100.0	0.0	10.9	45.0	0.0	8.2	96.7	3.3	10.8	96.7	0.0	7.6	56.7	0.0	358	61 [55-67]	14 [12-17]	82.5	0.6	83.1
C16:2	11.2	100.0	0.0	10.4	100.0	0.0	10.9	55.0	0.0	10.7	96.7	3.3	13.6	98.3	0.0	8.5	0.0	0.0	358	42 [33-46]	17 [11-22]	75.0	0.6	75.6
C16:2-OH	8.9	100.0	0.0	8.1	100.0	0.0	8.2	100.0	0.0	10.3	96.7	3.3	12.3	100.0	0.0	9.7	55.0	0.0	358	54 [50-59]	12 [7.3-17]	92.0	0.6	92.5
C18	5.9	1.7	0.0	8.4	0.0	0.0	7.4	0.0	0.0	7.7	38.3	3.3	7.1	0.0	0.0	9.6	0.0	0.0	358	12 [9.7-19]	12 [7.4-14]	6.7	0.6	7.2
C18:1	6.3	0.0	0.0	5.4	0.0	0.0	4.4	0.0	0.0	6.1	55.0	3.3	5.1	0.0	0.0	7.0	0.0	0.0	358	7.5 [5.8-11]	5.2 [3.7-8.7]	9.2	0.6	9.7
C18:1-OH	7.8	100.0	0.0	12.5	100.0	0.0	6.6	100.0	0.0	7.8	96.7	3.3	13.5	100.0	0.0	7.9	13.3	0.0	358	44 [36-49]	10 [3.7-18]	85.0	0.6	85.6
C18:2	7.5	0.0	0.0	8.0	0.0	0.0	5.6	0.0	0.0	9.8	36.7	3.3	7.1	0.0	0.0	8.8	0.0	0.0	358	11 [6.6-15]	11 [4.9-15]	6.1	0.6	6.7
C2	3.9	0.0	0.0	4.5	0.0	0.0	4.4	0.0	0.0	5.9	0.0	3.3	5.0	0.0	0.0	6.6	0.0	0.0	358	5.9 [3.9-8]	4.4 [3.4-7.1]	0.0	0.6	0.6
C3	4.6	0.0	0.0	5.6	0.0	0.0	5.2	0.0	0.0	5.5	0.0	3.3	6.4	0.0	0.0	7.8	0.0	0.0	358	16 [13-22]	8.5 [5.7-12]	0.0	0.6	0.6
C3:1	7.2	100.0	0.0	7.9	100.0	0.0	18.5	100.0	0.0	8.7	53.3	3.3	19.5	100.0	0.0	9.7	0.0	0.0	358	56 [50-63]	21 [12-29]	75.6	0.6	76.1
C3-DC (C4-OH)	6.0	63.3	0.0	7.1	10.0	0.0	6.7	75.0	0.0	6.8	20.0	3.3	9.0	5.0	0.0	12.2	0.0	0.0	358	33 [28-40]	13 [7.2-21]	28.9	0.6	29.4
C5-OH (C3-DC-M)	9.1	100.0	0.0	5.9	100.0	0.0	7.3	100.0	0.0	6.5	23.3	3.3	11.5	43.3	0.0	7.2	0.0	0.0	358	98 [82-120]	15 [8.2-22]	61.1	0.6	61.7
C3-OH	5.3	100.0	0.0	8.5	100.0	0.0	10.7	100.0	0.0	6.1	26.7	3.3	14.7	100.0	0.0	10.8	1.7	0.0	358	84 [71-100]	20 [11-28]	71.4	0.6	72.0
C4	5.9	0.0	0.0	6.8	0.0	0.0	5.9	0.0	0.0	7.3	0.0	3.3	7.3	0.0	0.0	7.0	0.0	0.0	358	11 [8.9-15]	9.1 [7.3-13]	0.0	0.6	0.6
C4:1	5.7	100.0	0.0	7.5	96.7	0.0	10.2	100.0	0.0	9.9	18.3	3.3	12.3	100.0	0.0	11.0	5.0	0.0	358	58 [49-64]	18 [14-28]	70.0	0.6	70.6
C5	5.1	0.0	0.0	5.9	0.0	0.0	5.9	8.3	0.0	7.2	0.0	3.3	3.5	0.0	0.0	8.8	0.0	0.0	358	25 [17-35]	12 [8-16]	1.4	0.6	1.9
C5:1	5.2	100.0	0.0	8.0	100.0	0.0	10.4	100.0	0.0	8.3	93.3	3.3	12.0	100.0	0.0	7.9	0.0	0.0	358	59 [51-66]	15 [9.6-25]	82.2	0.6	82.8
C5:1-DC	9.0	100.0	0.0	7.2	100.0	0.0	13.6	98.3	0.0	8.7	41.7	3.3	13.7	95.0	0.0	11.5	3.3	0.0	358	35 [25-46]	25 [12-35]	73.1	0.6	73.6
C5-DC (C6-OH)	10.1	96.7	0.0	7.1	80.0	0.0	10.0	90.0	0.0	10.9	35.0	3.3	10.0	85.0	0.0	8.2	0.0	0.0	358	35 [29-40]	17 [12-31]	64.5	0.6	65.0
C5-M-DC	8.4	100.0	0.0	9.5	100.0	0.0	10.4	100.0	0.0	6.0	96.7	3.3	11.5	100.0	0.0	7.2	100.0	0.0	358	53 [45-59]	9.9 [5-16]	99.5	0.6	100.0
C6:1	7.2	100.0	0.0	7.8	80.0	0.0	9.6	86.7	0.0	9.8	96.7	3.3	12.3	100.0	0.0	9.5	25.0	0.0	358	54 [46-71]	17 [11-52]	81.4	0.6	82.0
C6 (C4:1-DC)	6.2	98.3	0.0	6.2	0.0	0.0	6.2	81.7	0.0	7.0	1.7	3.3	6.4	45.0	0.0	7.6	0.0	0.0	358	52 [43-69]	12 [9.6-19]	37.8	0.6	38.3
C7-DC	6.4	93.3	0.0	8.2	73.3	0.0	8.5	31.7	0.0	9.9	0.0	3.3	9.1	45.0	0.0	10.5	0.0	0.0	358	18 [14-23]	13 [11-19]	40.6	0.6	41.1
C8	5.4	100.0	0.0	5.4	48.3	0.0	3.5	6.7	0.0	5.7	0.0	3.3	5.6	23.3	0.0	8.1	0.0	0.0	358	29 [23-33]	11 [8.7-16]	29.7	0.6	30.3
C9	6.1	80.0	0.0	6.2	100.0	0.0	6.3	18.3	0.0	7.4	38.3	3.3	8.8	8.3	0.0	6.0	0.0	0.0	358	52 [38-100]	29 [19-37]	40.8	0.6	41.4
lysoPC a C14:0	3.5	98.3	0.0	2.9	95.0	0.0	2.2	95.0	0.0	5.3	90.0	3.3	3.4	95.0	0.0	5.6	93.3	0.0	358	21 [18-26]	5.9 [4-7.8]	94.4	0.6	95.0
lysoPC a C16:0	5.6	0.0	0.0	5.9	0.0	0.0	4.0	0.0	0.0	8.0	0.0	3.3	4.6	0.0	0.0	7.7	0.0	0.0	358	19 [15-24]	7.7 [4.8-11]	0.0	0.6	0.6
lysoPC a C16:1	4.9	0.0	0.0	4.6	0.0	0.0	4.8	0.0	0.0	6.9	0.0	3.3	5.8	0.0	0.0	6.4	0.0	0.0	358	14 [12-18]	7.5 [5.3-10]	0.0	0.6	0.6
lysoPC a C17:0	5.2	0.0	0.0	5.2	0.0	0.0	6.4	0.0	0.0	8.6	0.0	3.3	5.7	0.0	0.0	8.2	0.0	0.0	358	15 [9.7-20]	11 [8.5-14]	0.0	0.6	0.6
lysoPC a C18:0	6.2	0.0	0.0	5.0	0.0	0.0	4.8	0.0	0.0															

Supplementary Table S-5

Analyte	LAB A			LAB B			LAB C			LAB D			LAB E			LAB F			Measurem. used	All labs					
	median CV	%<LOD	%missing	median CV	%<LOD	%missing	median CV	%<LOD	%missing	median CV	%<LOD	%missing	median CV	%<LOD	%missing	median CV	%<LOD	%missing		n	Median InterLab	Median InterLab	%<LOD	%missing	% total (BLD
																					%CV [90%CI] Non-Normalised	%CV [90%CI] Normalised			
lysoPC a C26:1	12.3	1.7	0.0	18.9	0.0	0.0	20.0	5.0	0.0	16.8	0.0	3.3	20.6	1.7	0.0	15.1	0.0	0.0	358	81 [68-88]	32 [24-44]	1.4	0.6	2.0	
lysoPC a C28:0	11.0	71.7	0.0	8.7	0.0	0.0	11.1	18.3	0.0	19.6	0.0	3.3	16.7	10.0	0.0	11.7	0.0	0.0	358	48 [36-55]	16 [9.9-24]	16.7	0.6	17.2	
lysoPC a C28:1	6.4	0.0	0.0	7.4	0.0	0.0	7.9	0.0	0.0	13.1	0.0	3.3	9.3	0.0	0.0	11.3	0.0	0.0	358	40 [35-49]	18 [14-24]	0.0	0.6	0.6	
PC aa C24:0	6.9	26.7	0.0	15.7	11.7	0.0	13.9	25.0	0.0	19.8	0.0	3.3	11.8	93.3	0.0	11.5	1.7	0.0	358	53 [45-67]	24 [18-47]	26.4	0.6	27.0	
PC aa C26:0	4.9	100.0	0.0	11.2	98.3	0.0	5.7	100.0	0.0	16.6	1.7	3.3	5.4	100.0	0.0	12.6	81.7	0.0	358	52 [44-61]	11 [6.4-18]	80.3	0.6	80.8	
PC aa C28:1	5.3	0.0	0.0	6.6	0.0	0.0	4.7	0.0	0.0	6.5	0.0	3.3	4.6	0.0	0.0	6.8	0.0	0.0	358	15 [12-16]	5.9 [3.9-8.7]	0.0	0.6	0.6	
PC aa C30:0	4.5	0.0	0.0	5.6	0.0	0.0	3.4	0.0	0.0	6.0	0.0	3.3	5.4	0.0	0.0	6.9	0.0	0.0	358	9.4 [6.7-11]	4.3 [3.4-6.2]	0.0	0.6	0.6	
PC aa C30:2	59.1	3.3	28.3	38.7	0.0	1.7	5.9	0.0	0.0	71.7	0.0	63.3	51.7	0.0	8.3	NQ	0.0	100.0	239	160 [150-170]	31 [10-59]	0.6	33.6	34.2	
PC aa C32:0	5.6	0.0	0.0	5.2	0.0	0.0	3.8	0.0	0.0	5.8	0.0	3.3	4.7	0.0	0.0	7.0	0.0	0.0	358	18 [16-20]	6.3 [3.6-7.4]	0.0	0.6	0.6	
PC aa C32:1	5.3	0.0	0.0	4.6	0.0	0.0	3.4	0.0	0.0	9.6	0.0	3.3	4.5	0.0	0.0	6.7	0.0	0.0	358	23 [20-24]	5.2 [3.5-7.7]	0.0	0.6	0.6	
PC aa C32:2	4.5	0.0	0.0	6.0	0.0	0.0	4.3	0.0	0.0	7.2	0.0	3.3	5.1	0.0	0.0	NQ	0.0	100.0	298	19 [16-20]	5 [3.3-7.5]	0.0	17.2	17.2	
PC aa C32:3	6.1	0.0	0.0	6.3	0.0	0.0	4.7	0.0	0.0	6.7	0.0	3.3	3.7	0.0	0.0	7.0	0.0	0.0	358	19 [16-21]	6.6 [4-8.6]	0.0	0.6	0.6	
PC aa C34:1	4.7	0.0	0.0	6.1	0.0	0.0	3.8	0.0	0.0	5.1	0.0	3.3	4.5	0.0	0.0	7.2	0.0	0.0	358	29 [23-32]	6.8 [4.5-16]	0.0	0.6	0.6	
PC aa C34:2	4.7	0.0	0.0	4.5	0.0	0.0	3.7	0.0	0.0	5.3	0.0	3.3	4.8	0.0	0.0	7.5	0.0	0.0	358	45 [43-51]	7.2 [5-15]	0.0	0.6	0.6	
PC aa C34:3	5.2	0.0	0.0	4.6	0.0	0.0	4.4	0.0	0.0	5.3	0.0	3.3	5.4	0.0	0.0	7.0	0.0	0.0	358	20 [16-22]	4.7 [3.1-9.2]	0.0	0.6	0.6	
PC aa C34:4	5.2	0.0	0.0	5.0	0.0	0.0	4.3	0.0	0.0	7.3	0.0	3.3	4.4	0.0	0.0	6.9	0.0	0.0	358	20 [18-22]	5.4 [3.6-7.2]	0.0	0.6	0.6	
PC aa C36:0	5.4	0.0	0.0	7.4	0.0	0.0	4.4	0.0	0.0	14.6	0.0	3.3	7.4	0.0	0.0	6.7	0.0	0.0	358	21 [16-32]	28 [9.2-38]	0.0	0.6	0.6	
PC aa C36:1	5.6	0.0	0.0	5.0	0.0	0.0	3.6	0.0	0.0	6.3	0.0	3.3	5.4	0.0	0.0	7.2	0.0	0.0	358	15 [11-19]	5.2 [4-8.4]	0.0	0.6	0.6	
PC aa C36:2	4.1	0.0	0.0	4.4	0.0	0.0	4.2	0.0	0.0	5.8	0.0	3.3	4.3	0.0	0.0	7.5	0.0	0.0	358	33 [29-35]	6.4 [4-11]	0.0	0.6	0.6	
PC aa C36:3	5.0	0.0	0.0	5.5	0.0	0.0	4.1	0.0	0.0	5.3	0.0	3.3	4.4	0.0	0.0	6.6	0.0	0.0	358	21 [18-23]	8.2 [5.6-11]	0.0	0.6	0.6	
PC aa C36:4	4.7	0.0	0.0	4.9	0.0	0.0	3.9	0.0	0.0	5.0	0.0	3.3	4.7	0.0	0.0	6.9	0.0	0.0	358	24 [20-30]	7.6 [4.2-17]	0.0	0.6	0.6	
PC aa C36:5	5.0	0.0	0.0	5.4	0.0	0.0	3.6	0.0	0.0	6.0	0.0	3.3	4.3	0.0	0.0	7.4	0.0	0.0	358	18 [15-21]	4.9 [3.1-7.9]	0.0	0.6	0.6	
PC aa C36:6	4.6	0.0	0.0	5.4	0.0	0.0	4.3	0.0	0.0	7.2	0.0	3.3	5.4	0.0	0.0	7.6	0.0	0.0	358	23 [19-26]	7.3 [5.8-14]	0.0	0.6	0.6	
PC aa C38:0	5.2	0.0	0.0	5.1	0.0	0.0	4.5	0.0	0.0	6.0	0.0	3.3	3.5	0.0	0.0	7.6	0.0	0.0	358	10 [8.9-12]	5.6 [3.8-7.6]	0.0	0.6	0.6	
PC aa C38:1	19.8	0.0	0.0	15.4	0.0	0.0	13.1	0.0	0.0	25.1	0.0	3.3	17.9	0.0	0.0	NQ	0.0	100.0	298	18 [9.9-24]	14 [9.2-22]	0.0	17.2	17.2	
PC aa C38:3	3.6	0.0	0.0	5.0	0.0	0.0	4.1	0.0	0.0	6.5	0.0	3.3	4.5	0.0	0.0	8.0	0.0	0.0	358	6.1 [4.9-8.2]	6.1 [4-9.6]	0.0	0.6	0.6	
PC aa C38:4	3.6	0.0	0.0	4.5	0.0	0.0	4.1	0.0	0.0	5.0	0.0	3.3	4.6	0.0	0.0	7.4	0.0	0.0	358	12 [10-15]	7 [3.8-9.4]	0.0	0.6	0.6	
PC aa C38:5	4.4	0.0	0.0	4.8	0.0	0.0	3.7	0.0	0.0	6.9	0.0	3.3	3.9	0.0	0.0	6.8	0.0	0.0	358	12 [10-14]	5.4 [3.4-6.4]	0.0	0.6	0.6	
PC aa C38:6	4.4	0.0	0.0	4.8	0.0	0.0	4.0	0.0	0.0	7.6	0.0	3.3	4.3	0.0	0.0	8.1	0.0	0.0	358	14 [12-18]	6.1 [4.1-12]	0.0	0.6	0.6	
PC aa C40:1	6.1	28.3	0.0	5.0	28.3	0.0	5.0	18.3	0.0	7.9	95.0	3.3	6.1	41.7	0.0	6.3	60.0	0.0	358	12 [8.9-13]	5.2 [3.4-7]	45.3	0.6	45.8	
PC aa C40:2	7.0	0.0	0.0	7.9	0.0	0.0	7.7	0.0	0.0	9.1	0.0	3.3	6.3	0.0	0.0	7.0	0.0	0.0	358	13 [11-17]	7.8 [4.2-13]	0.0	0.6	0.6	
PC aa C40:3	6.6	0.0	0.0	6.7	0.0	0.0	6.4	0.0	0.0	8.9	0.0	3.3	6.2	0.0	0.0	6.5	0.0	0.0	358	13 [10-17]	8.2 [5.4-12]	0.0	0.6	0.6	
PC aa C40:4	4.5	0.0	0.0	5.8	0.0	0.0	4.4	0.0	0.0	8.2	0.0	3.3	5.8	0.0	0.0	6.8	0.0	0.0	358	11 [10-13]	4.9 [4.1-6.4]	0.0	0.6	0.6	
PC aa C40:5	4.9	0.0	0.0	5.9	0.0	0.0	4.2	0.0	0.0	6.2	0.0	3.3	4.5	0.0	0.0	7.0	0.0	0.0	358	14 [13-16]	4 [2.3-7.5]	0.0	0.6	0.6	
PC aa C40:6	4.8	0.0	0.0	5.1	0.0	0.0	3.7	0.0	0.0	5.2	0.0	3.3	4.3	0.0	0.0	7.5	0.0	0.0	358	11 [10-14]	4.3 [2.8-6.5]	0.0	0.6	0.6	
PC aa C42:0	6.1	0.0	0.0	6.4	0.0	0.0	4.8	0.0	0.0	7.6	0.0	3.3	5.1	0.0	0.0	7.9	0.0	0.0	358	12 [9.5-15]	5.4 [3.2-8.6]	0.0	0.6	0.6	
PC aa C42:1	4.7	0.0	0.0	6.5	0.0	0.0	8.2	0.0	0.0	9.6	0.0	3.3	4.3	0.0	0.0	6.9	0.0	0.0	358	11 [9.3-13]	5.7 [3.5-9.7]	0.0	0.6	0.6	
PC aa C42:2	6.0	0.0	0.0	5.8	0.0	0.0	6.1	0.0	0.0	8.4	0.0	3.3	7.2	0.0	0.0	6.7	0.0	0.0	358	15 [13-17]	6.8 [4.5-9.1]	0.0	0.6	0.6	
PC aa C42:4	9.4	0.0	0.0	10.7	0.0	0.0	8.9	0.0	0.0	10.6	0.0	3.3	6.9	0.0	0.0	6.8	0.0	0.0	358	25 [18-28]	7.8 [5.8-13]	0.0	0.6	0.6	
PC aa C42:5	6.7	0.0	0.0	8.1	0.0	0.0	6.8	0.0	0.0	8.4	0.0	3.3	6.6	0.0	0.0	7.7	0.0	0.0	358	14 [13-17]	6.8 [4.5-9.4]	0.0	0.6	0.6	
PC aa C42:6	5.3	0.0	0.0	6.0	0.0	0.0	5.5	0.0	0.0	8.8	81.7	3.3	5.1	0.0	0.0	5.4	0.0	0.0	358	13 [11-15]	6.8 [4.6-9.3]	13.6	0.6	14.2	
PC ae C30:0	4.8	0.0	0.0	5.6	0.0	0.0	7.2	0.0	0.0	7.6	0.0	3.3	4.9	0.0	0.0	7.1	0.0	0.0	358	14 [10-15]	7.6 [4.7-10]	0.0	0.6	0.6	
PC ae C30:1	41.8	1.7	35.0	27.3	0.0	0.0	16.1	0.0	0.0	25.7	0.0	3.3	23.9	0.0	1.7	43.0	0.0	56.7	302	53 [40-74]	56 [25-160]	0.3	16.1	16.4	
PC ae C30:2	6.3	0.0	0.0	8.9	0.0	0.0	6.8	0.0	0.0	9.4	0.0	3.3	8.3	0.0	0.0	8.8	0.0	0.0	358	29 [26-32]	7.9 [5.4-13]	0.0	0.6	0.6	
PC ae C32:1	6.5	0.0	0.0	5.0	0.0	0.0	4.4	0.0	0.0	7.0	0.0	3.3	4.8	0.0	0.0	7.6	0.0	0.0	358						

Supplementary Table S-5

Analyte	LAB A			LAB B			LAB C			LAB D			LAB E			LAB F			Measur.	All labs				
	median CV	%<LOD	%missing	median CV	%<LOD	%missing	median CV	%<LOD	%missing	median CV	%<LOD	%missing	median CV	%<LOD	%missing	median CV	%<LOD	%missing	n	Median InterLab	Median InterLab	%<LOD	%missing	% total (BLD +missing)
																				CV [90%CI]	CV [90%CI]			
																				Non-Normalised	Normalised			
PC ae C36:1	4.5	0.0	0.0	5.3	0.0	0.0	3.4	0.0	0.0	6.8	0.0	3.3	4.3	0.0	0.0	7.4	0.0	0.0	358	20 [17-22]	6.7 [5.1-11]	0.0	0.6	0.6
PC ae C36:2	4.7	0.0	0.0	5.2	0.0	0.0	3.3	0.0	0.0	6.0	0.0	3.3	4.3	0.0	0.0	7.8	0.0	0.0	358	15 [13-16]	4.6 [3.2-6.4]	0.0	0.6	0.6
PC ae C36:3	5.2	0.0	0.0	4.7	0.0	0.0	4.0	0.0	0.0	6.7	0.0	3.3	4.1	0.0	0.0	7.7	0.0	0.0	358	19 [16-21]	5.7 [4.1-7.5]	0.0	0.6	0.6
PC ae C36:4	4.9	0.0	0.0	4.4	0.0	0.0	3.7	0.0	0.0	6.7	0.0	3.3	4.4	0.0	0.0	8.4	0.0	0.0	358	17 [15-19]	5.9 [4.2-7.5]	0.0	0.6	0.6
PC ae C36:5	5.8	0.0	0.0	5.1	0.0	0.0	4.0	0.0	0.0	7.1	0.0	3.3	5.0	0.0	0.0	8.3	0.0	0.0	358	14 [12-16]	5.3 [4-7.3]	0.0	0.6	0.6
PC ae C38:0	4.8	0.0	0.0	4.3	0.0	0.0	3.5	0.0	0.0	7.4	0.0	3.3	4.4	0.0	0.0	7.7	0.0	0.0	358	14 [10-16]	5.8 [3.3-10]	0.0	0.6	0.6
PC ae C38:1	37.1	0.0	10.0	19.8	0.0	0.0	20.2	0.0	0.0	26.4	1.7	11.7	13.1	0.0	0.0	NA	0.0	81.7	298	49 [27-91]	32 [18-43]	0.3	17.2	17.5
PC ae C38:2	5.8	0.0	0.0	6.2	0.0	0.0	5.4	0.0	0.0	13.4	0.0	3.3	6.4	0.0	0.0	9.9	0.0	0.0	358	30 [26-39]	15 [6.8-25]	0.0	0.6	0.6
PC ae C38:3	4.7	0.0	0.0	4.9	0.0	0.0	4.4	0.0	0.0	5.2	0.0	3.3	3.5	0.0	0.0	7.4	0.0	0.0	358	14 [12-19]	7.9 [5.8-12]	0.0	0.6	0.6
PC ae C38:4	5.0	0.0	0.0	4.4	0.0	0.0	4.5	0.0	0.0	5.4	0.0	3.3	5.1	0.0	0.0	7.5	0.0	0.0	358	13 [11-14]	4.9 [3.3-6.6]	0.0	0.6	0.6
PC ae C38:5	4.7	0.0	0.0	5.0	0.0	0.0	4.3	0.0	0.0	6.2	0.0	3.3	4.8	0.0	0.0	7.9	0.0	0.0	358	13 [11-15]	5.7 [4.4-7.3]	0.0	0.6	0.6
PC ae C38:6	5.6	0.0	0.0	5.5	0.0	0.0	4.3	0.0	0.0	6.4	0.0	3.3	3.8	0.0	0.0	8.0	0.0	0.0	358	12 [11-15]	5.1 [4.3-7.7]	0.0	0.6	0.6
PC ae C40:1	6.4	0.0	0.0	4.9	0.0	0.0	5.1	0.0	0.0	12.1	0.0	3.3	4.6	0.0	0.0	6.4	0.0	0.0	358	13 [10-15]	8.4 [5.6-11]	0.0	0.6	0.6
PC ae C40:2	5.1	0.0	0.0	4.6	0.0	0.0	4.4	0.0	0.0	7.3	0.0	3.3	4.9	0.0	0.0	7.1	0.0	0.0	358	12 [8.2-14]	6.5 [4.4-8.6]	0.0	0.6	0.6
PC ae C40:3	4.1	0.0	0.0	4.7	0.0	0.0	5.7	0.0	0.0	8.6	0.0	3.3	5.6	0.0	0.0	6.6	0.0	0.0	358	21 [16-23]	11 [8-13]	0.0	0.6	0.6
PC ae C40:4	4.7	0.0	0.0	5.3	0.0	0.0	4.3	0.0	0.0	6.4	0.0	3.3	6.1	0.0	0.0	7.6	0.0	0.0	358	9.1 [7.6-13]	5.8 [3.3-8.1]	0.0	0.6	0.6
PC ae C40:5	4.2	0.0	0.0	4.8	0.0	0.0	4.7	0.0	0.0	7.5	0.0	3.3	4.3	0.0	0.0	8.0	0.0	0.0	358	10 [8.7-13]	5.2 [3-8.5]	0.0	0.6	0.6
PC ae C40:6	4.5	0.0	0.0	5.0	0.0	0.0	3.9	0.0	0.0	7.1	0.0	3.3	4.5	0.0	0.0	7.0	0.0	0.0	358	13 [10-15]	5.9 [4.4-7.5]	0.0	0.6	0.6
PC ae C42:0	3.2	98.3	0.0	4.5	78.3	0.0	3.3	76.7	0.0	4.0	96.7	3.3	3.5	100.0	0.0	4.1	86.7	0.0	358	30 [24-34]	6.2 [4.4-11]	89.5	0.6	90.0
PC ae C42:1	4.9	0.0	0.0	8.5	51.7	0.0	6.5	0.0	0.0	13.4	0.0	3.3	9.3	0.0	0.0	5.7	0.0	0.0	358	15 [12-19]	7 [3.7-10]	8.6	0.6	9.2
PC ae C42:2	4.5	0.0	0.0	6.7	0.0	0.0	6.5	0.0	0.0	10.5	0.0	3.3	4.7	0.0	0.0	7.3	0.0	0.0	358	11 [9.5-14]	6.5 [4.9-9.1]	0.0	0.6	0.6
PC ae C42:3	6.3	0.0	0.0	7.2	0.0	0.0	4.8	0.0	0.0	10.1	0.0	3.3	6.2	0.0	0.0	5.7	0.0	0.0	358	8.2 [6.4-12]	7.4 [5.1-8.7]	0.0	0.6	0.6
PC ae C42:4	5.8	0.0	0.0	6.6	0.0	0.0	5.1	0.0	0.0	9.0	0.0	3.3	5.2	0.0	0.0	7.7	0.0	0.0	358	12 [10-14]	5.5 [4.4-8.7]	0.0	0.6	0.6
PC ae C42:5	3.8	0.0	0.0	4.2	0.0	0.0	5.1	0.0	0.0	6.3	0.0	3.3	5.1	0.0	0.0	6.2	0.0	0.0	358	9.8 [8.7-11]	4.7 [2.7-8.2]	0.0	0.6	0.6
PC ae C44:3	8.2	0.0	0.0	6.9	0.0	0.0	11.6	0.0	0.0	14.0	0.0	3.3	12.0	0.0	0.0	5.9	0.0	0.0	358	19 [14-23]	12 [8.1-19]	0.0	0.6	0.6
PC ae C44:4	6.2	0.0	0.0	7.4	0.0	0.0	6.0	0.0	0.0	10.7	0.0	3.3	6.0	0.0	0.0	5.6	0.0	0.0	358	16 [14-17]	5.2 [4.3-6.5]	0.0	0.6	0.6
PC ae C44:5	5.1	0.0	0.0	6.1	0.0	0.0	6.1	0.0	0.0	6.7	0.0	3.3	4.9	0.0	0.0	6.5	0.0	0.0	358	19 [18-21]	4.8 [3.2-7]	0.0	0.6	0.6
PC ae C44:6	3.6	0.0	0.0	4.4	0.0	0.0	3.3	0.0	0.0	6.6	0.0	3.3	4.7	0.0	0.0	7.9	0.0	0.0	358	16 [14-19]	5.8 [4.3-7]	0.0	0.6	0.6
SM (OH) C14:1	4.5	0.0	0.0	4.7	0.0	0.0	4.7	0.0	0.0	7.6	0.0	3.3	4.9	0.0	0.0	6.7	0.0	0.0	358	30 [28-35]	5.3 [3-8.7]	0.0	0.6	0.6
SM (OH) C16:1	4.9	0.0	0.0	5.3	0.0	0.0	5.0	0.0	0.0	7.1	0.0	3.3	4.8	0.0	0.0	7.9	0.0	0.0	358	39 [37-44]	5.6 [2.7-8.3]	0.0	0.6	0.6
SM (OH) C22:1	5.9	0.0	0.0	4.5	0.0	0.0	4.6	0.0	0.0	8.0	0.0	3.3	5.4	0.0	0.0	6.9	0.0	0.0	358	100 [97-110]	7.7 [3.9-10]	0.0	0.6	0.6
SM (OH) C22:2	6.6	0.0	0.0	4.3	0.0	0.0	5.1	0.0	0.0	8.7	0.0	3.3	4.0	0.0	0.0	7.3	0.0	0.0	358	120 [120-130]	6.7 [3.4-10]	0.0	0.6	0.6
SM (OH) C24:1	7.9	0.0	0.0	7.7	0.0	0.0	6.5	0.0	0.0	7.0	0.0	3.3	7.1	0.0	0.0	7.4	0.0	0.0	358	63 [57-67]	8.2 [5.7-11]	0.0	0.6	0.6
SM C16:0	4.6	0.0	0.0	4.6	0.0	0.0	4.2	0.0	0.0	6.6	0.0	3.3	4.5	0.0	0.0	6.8	0.0	0.0	358	36 [28-43]	5.2 [2.5-8.9]	0.0	0.6	0.6
SM C16:1	5.4	0.0	0.0	5.3	0.0	0.0	4.7	0.0	0.0	7.3	0.0	3.3	4.2	0.0	0.0	6.4	0.0	0.0	358	39 [36-44]	5.1 [2.6-8.3]	0.0	0.6	0.6
SM C18:0	4.5	0.0	0.0	4.7	0.0	0.0	3.8	0.0	0.0	7.0	0.0	3.3	4.8	0.0	0.0	7.4	0.0	0.0	358	50 [45-56]	6.1 [3.9-8.4]	0.0	0.6	0.6
SM C18:1	5.1	0.0	0.0	5.2	0.0	0.0	3.8	0.0	0.0	5.7	0.0	3.3	5.1	0.0	0.0	5.7	0.0	0.0	358	45 [41-51]	4.9 [3-8.1]	0.0	0.6	0.6
SM C20:2	8.8	0.0	0.0	11.3	0.0	0.0	5.1	0.0	0.0	11.9	0.0	3.3	13.1	0.0	0.0	7.5	0.0	0.0	358	110 [98-120]	13 [8.6-25]	0.0	0.6	0.6
SM C22:3	59.0	0.0	90.0	60.4	1.7	65.0	5.0	0.0	0.0	9.4	0.0	85.0	131.9	0.0	91.7	NQ	0.0	100.0	101	170 [170-170]	51 [35-67]	0.3	72.0	72.2
SM C24:0	5.6	0.0	0.0	4.7	0.0	0.0	4.4	0.0	0.0	6.6	0.0	3.3	5.0	0.0	0.0	7.2	0.0	0.0	358	70 [66-77]	6.3 [3.8-10]	0.0	0.6	0.6
SM C24:1	5.9	0.0	0.0	4.9	0.0	0.0	4.6	0.0	0.0	7.4	0.0	3.3	4.8	0.0	0.0	8.0	0.0	0.0	358	110 [100-120]	7.1 [3.5-11]	0.0	0.6	0.6
SM C26:0	10.2	0.0	0.0	20.1	0.0	0.0	16.8	0.0	0.0	19.7	0.0	3.3	10.8	0.0	0.0	11.0	0.0	0.0	358	78 [73-88]	14 [7.7-21]	0.0	0.6	0.6
SM C26:1	8.0	0.0	0.0	11.2	0.0	0.0	7.2	0.0	0.0	15.0	0.0	3.3	11.8	0.0	0.0	8.4	0.0	0.0	358	68 [60-74]	12 [9.8-16]	0.0	0.6	0.6
H1	4.5	0.0	0.0	5.2	0.0	0.0	4.4	0.0	0.0	6.5	0.0	3.3	4.3	0.0	0.0	11.5	0.0	1.7	357	9.3 [7.5-16]	6.3 [3.9-13]	0.0	0.8	0.8
Ala	1.8	0.0	0.0	2.1	0.0	0.0	3.3	0.0	0.0	4.1	0.0	3.3	4.2	0.0	0.0	4.5	0.0	6.7	354	3.6 [2.7-6.8]		0.0	1.7	1.7
Arg	5.0	0.0	0.0	5.7	0.0	0.0	5.1	0.0	0.0	6.2	0.0	3.3	8.5	0.0	0.0	8.3	0.0	0.0	358	7.1 [4.1-22]		0.0	0.6	0.6
Asn	3.4	0.0	0.0	2.4	0.0	0.0	4.6	0.0	0.0	4.4	0.0	3.3	6.1	0.0	0.0	6.4	0.0	6.7	354	6.8 [4-10]		0.0	1.7	1.7
Asp	11.2	0.0	1.7	10.8	0.0	0.0	21.6	0.0	0.0	24.2	21.7	30.0	14.3	0.0	0.0	17.4	0.0	6.7	337	38 [13-59]		3.6	6.4	10.0
Cit	4.0	0.0	0.0	3.2	0.0	0.0	5.8	0.0	0.0	5.8	0.0	3.3	11.6	0.0	0.0	6.5	0.0	6.7	354	9.3 [6.2-12]		0.0	1.7	1.7
Gln	5.0	0.0	0.0	4.7	0.0	0.0	5.6	0.0	0.0	7.9	0.0	3.3	8.5	0.0	0.0	6.6	0.0	0.0	358	8.3 [6.5-11]		0.0	0.6	0.6
Glu	4.1	0.0	0.0	3.1	0.0	0.0	6.6	0.0	0.0	6.6	0.0	3.3	8.4	0.0	0.0	6.8	0.0	6.7	354	45 [4.8-87]		0.0	1.7	1.7

Supplementary Table S-5

Analyte	LAB A			LAB B			LAB C			LAB D			LAB E			LAB F			Measur- used	All labs				
	median CV	%<LOD	%missing	median CV	%<LOD	%missing	median CV	%<LOD	%missing	median CV	%<LOD	%missing	median CV	%<LOD	%missing	median CV	%<LOD	%missing	n	Median InterLab %CV [90%CI] Non-Normalised	Median InterLab %CV [90%CI] Normalised	%<LOD	%missing	% total (BLD +missing)
Gly	4.4	0.0	0.0	4.5	0.0	0.0	6.5	0.0	0.0	6.1	0.0	3.3	5.5	0.0	0.0	7.1	0.0	0.0	358	5.9 [4.9-9.6]		0.0	0.6	0.6
His	4.5	0.0	0.0	5.8	0.0	0.0	5.6	0.0	0.0	5.9	0.0	3.3	4.8	0.0	0.0	6.8	0.0	0.0	358	4.5 [3.3-6.8]		0.0	0.6	0.6
Ile	3.7	0.0	0.0	5.2	0.0	0.0	5.2	0.0	0.0	6.1	0.0	3.3	4.0	0.0	0.0	6.9	0.0	0.0	358	9.6 [7.4-11]		0.0	0.6	0.6
Leu	4.0	0.0	0.0	4.1	0.0	0.0	5.8	0.0	0.0	7.0	0.0	3.3	4.8	0.0	0.0	7.9	0.0	0.0	358	9.6 [8-10]		0.0	0.6	0.6
Lys	4.8	0.0	0.0	5.3	0.0	0.0	6.8	0.0	0.0	6.4	0.0	3.3	6.3	0.0	0.0	6.6	0.0	0.0	358	13 [10-16]		0.0	0.6	0.6
Met	4.8	0.0	0.0	4.8	0.0	0.0	7.0	0.0	0.0	6.2	0.0	3.3	5.8	0.0	0.0	7.0	0.0	0.0	358	6.5 [4.5-9.5]		0.0	0.6	0.6
Orn	4.4	0.0	0.0	5.6	0.0	0.0	6.1	0.0	0.0	7.6	0.0	3.3	8.2	0.0	0.0	7.3	0.0	0.0	358	8.4 [5.5-11]		0.0	0.6	0.6
Phe	3.7	0.0	0.0	5.2	0.0	0.0	5.0	0.0	0.0	6.0	0.0	3.3	4.1	0.0	0.0	9.1	0.0	0.0	358	7.5 [6-11]		0.0	0.6	0.6
Pro	4.6	0.0	0.0	5.0	0.0	0.0	5.8	0.0	0.0	5.3	0.0	3.3	4.5	0.0	0.0	7.9	0.0	0.0	358	9.9 [9-16]		0.0	0.6	0.6
Ser	4.7	0.0	0.0	5.1	0.0	0.0	7.5	0.0	0.0	8.3	0.0	3.3	8.8	0.0	0.0	7.3	0.0	0.0	358	6.2 [3.6-12]		0.0	0.6	0.6
Thr	2.2	0.0	0.0	2.4	0.0	0.0	4.1	0.0	0.0	3.7	0.0	3.3	8.4	0.0	0.0	4.6	0.0	6.7	354	5.6 [3.5-8.7]		0.0	1.7	1.7
Trp	4.7	0.0	0.0	4.5	0.0	0.0	6.0	0.0	0.0	6.4	0.0	3.3	5.6	0.0	0.0	8.1	0.0	0.0	358	4.5 [2.5-7.8]		0.0	0.6	0.6
Tyr	3.9	0.0	0.0	5.0	0.0	0.0	7.0	0.0	0.0	7.5	0.0	3.3	4.7	0.0	0.0	8.3	0.0	0.0	358	4.8 [2.6-8.4]		0.0	0.6	0.6
Val	4.1	0.0	0.0	4.2	0.0	0.0	5.6	0.0	0.0	6.1	0.0	3.3	4.8	0.0	0.0	7.2	0.0	0.0	358	7.1 [5.4-9.4]		0.0	0.6	0.6
Ac-Orn	NA	0.0	100.0	4.7	0.0	0.0	7.6	0.0	0.0	10.1	53.3	3.3	6.6	0.0	0.0	2.8	96.7	3.3	296	56 [48-63]		25.0	17.8	42.8
ADMA	14.2	0.0	0.0	5.4	0.0	0.0	11.2	0.0	0.0	12.7	0.0	5.0	17.9	0.0	0.0	19.7	0.0	6.7	353	31 [25-37]		0.0	2.0	2.0
alpha-AAA	11.6	0.0	0.0	10.0	0.0	0.0	15.9	0.0	0.0	42.7	95.0	3.3	12.2	0.0	0.0	13.6	0.0	0.0	358	26 [15-40]		15.8	0.6	16.4
c4-OH-Pro	NA	0.0	100.0	NA	0.0	100.0	NA	0.0	100.0	6.0	0.0	3.3	9.1	96.7	3.3	NA	0.0	100.0	116	NA [NA-NA]		16.1	67.8	83.9
Carnosine	3.5	0.0	66.7	8.9	90.0	0.0	NA	0.0	100.0	0.5	93.3	6.7	5.1	0.0	96.7	NA	0.0	100.0	138	140 [130-140]		30.6	61.7	92.2
Creatinine	1.8	0.0	0.0	2.6	0.0	0.0	2.6	0.0	0.0	3.7	0.0	3.3	3.5	0.0	0.0	49.7	0.0	6.7	354	13 [3.6-25]		0.0	1.7	1.7
DOPA	0.7	0.0	91.7	4.0	100.0	0.0	NA	0.0	100.0	3.4	76.7	23.3	50.1	0.0	61.7	2.9	11.7	88.3	141	80 [62-100]		31.4	60.8	92.2
Dopamine	NA	0.0	100.0	NA	0.0	100.0	NA	0.0	100.0	10.4	95.0	5.0	NA	0.0	100.0	NA	0.0	100.0	57	NA [NA-NA]		15.8	84.2	100.0
Histamine	0.1	98.3	1.7	0.1	100.0	0.0	0.3	0.0	0.0	NA	0.0	100.0	0.3	98.3	1.7	NA	0.0	96.7	240	34 [33-35]		49.4	33.4	82.8
Kynurenine	3.8	0.0	0.0	5.5	0.0	0.0	6.7	0.0	0.0	6.9	0.0	3.3	6.7	0.0	0.0	7.1	0.0	0.0	358	5.6 [4.8-6]		0.0	0.6	0.6
Met-SO	12.1	0.0	0.0	7.5	0.0	0.0	8.1	0.0	0.0	9.0	90.0	3.3	18.2	0.0	78.3	48.9	0.0	33.3	291	61 [16-78]		15.0	19.2	34.2
Nitro-Tyr	1.6	0.0	90.0	NA	0.0	100.0	NA	0.0	100.0	16.4	96.7	3.3	NA	0.0	100.0	NA	1.7	98.3	65	NA [NA-NA]		16.4	81.9	98.3
PEA	NA	0.0	100.0	NA	0.0	100.0	NA	0.0	100.0	49.8	65.0	8.3	NA	0.0	100.0	NA	0.0	100.0	55	NA [NA-NA]		10.8	84.7	95.6
Putrescine	14.7	11.7	0.0	10.3	36.7	0.0	7.1	0.0	0.0	NA	0.0	100.0	15.3	0.0	10.0	11.4	0.0	55.0	261	27 [16-54]		8.1	27.5	35.6
Sarcosine	NQ	0.0	100.0	NQ	0.0	100.0	NQ	0.0	100.0	8.9	0.0	58.3	NQ	0.0	100.0	6.1	0.0	6.7	81	NA [NA-NA]		0.0	77.5	77.5
SDMA	64.5	0.0	55.0	7.7	0.0	0.0	19.6	0.0	0.0	7.2	0.0	3.3	26.9	0.0	0.0	9.2	0.0	6.7	321	140 [110-150]		0.0	10.8	10.8
Serotonin	4.1	0.0	0.0	6.4	5.0	0.0	5.1	0.0	0.0	8.3	0.0	6.7	7.1	0.0	0.0	6.9	0.0	6.7	352	9.9 [4.9-48]		0.8	2.2	3.1
Spermidine	1.6	0.0	0.0	1.6	75.0	0.0	3.1	0.0	0.0	7.0	0.0	3.3	2.8	0.0	0.0	4.4	0.0	6.7	354	56 [53-59]		12.5	1.7	14.2
Spermine	1.3	0.0	0.0	1.1	95.0	0.0	1.5	0.0	0.0	21.4	0.0	75.0	2.9	0.0	0.0	5.7	0.0	41.7	290	25 [23-55]		15.8	19.5	35.3
t4-OH-Pro	3.7	0.0	0.0	6.5	0.0	0.0	7.7	0.0	0.0	6.2	0.0	3.3	7.9	0.0	0.0	8.8	0.0	0.0	358	6.9 [4.8-11]		0.0	0.6	0.6
Taurine	2.8	0.0	0.0	1.6	0.0	0.0	3.9	0.0	0.0	3.9	0.0	3.3	6.2	0.0	0.0	5.6	0.0	6.7	354	6.2 [4.1-9.1]		0.0	1.7	1.7
total DMA	4.7	0.0	0.0	3.2	0.0	0.0	8.3	0.0	0.0	NQ	0.0	100.0	17.3	0.0	0.0	NQ	0.0	100.0	240	15 [9-20]		0.0	33.3	33.3

Supplementary Table S-6. Median intra-laboratory %CV, median inter-laboratory %CV (prior and post normalisation), and per laboratory % BLD and % missing values, for the 20 test material and 189 metabolites, per metabolite class. The column indicating missing includes the total of not acquired data 'NQ', 'NA' values, zero values and outliers.

Supplementary Table S-6

Metabolite class	LAB A			LAB B			LAB C			LAB D			LAB E			LAB F			Measurem . used	All labs				
	median CV	%<LOD	%missing	median CV	%<LOD	%missing	median CV	%<LOD	%missing	median CV	%<LOD	%missing	median CV	%<LOD	%missing	median CV	%<LOD	%missing	n	Median InterLab %CV [90%CI] Non-Normalised	Median InterLab %CV [90%CI]	%<LOD	%missing	% total (BLD +missing)
Amino Acids	4.4 [3.4-5]	0.0	0.1	4.8 [2.4-5.7]	0.0	0.0	5.8 [4.6-7]	0.0	0.0	6.2 [4.3-7.6]	1.0	4.6	5.8 [4.2-8.8]	0.0	0.0	7.3 [6.7-8.3]	0.0	1.9	7477	7.1 [4.5-13]		0.2	1.1	1.3
Biogenic Amines	3.7 [1-14]	5.0	36.6	5.4 [1.4-9.3]	22.8	22.7	7.1 [2-14]	0.0	31.8	8.3 [3.6-26]	30.2	23.8	7.5 [2.9-21]	8.9	29.6	7.1 [3.5-37]	5.0	43.8	5433	27 [6.6-100]		12.0	31.4	43.4
Carnitines	6.4 [4.9-8.9]	68.0	0.0	7.1 [5.3-9]	55.0	0.0	6.6 [4.4-11]	44.9	0.0	7.7 [6-10]	50.6	3.3	9.1 [5.1-14]	50.7	0.0	8 [6.5-11]	15.4	0.0	14320	34 [11-61]	12 [7.2-18]	47.4	0.6	48.0
Di-acyl PC (PC aa)	5.2 [4.4-6.9]	4.2	0.7	5.7 [4.6-11]	3.6	0.0	4.3 [3.6-7.9]	3.8	0.0	7.2 [5.3-15]	4.7	4.9	4.9 [4.3-7.3]	6.2	0.2	7 [6.6-7.9]	3.8	7.9	13365	17 [11-36]	6.4 [4.8-12]	4.4	2.3	6.7
Alkyl-acyl PC (PC ae)	5.1 [4.2-6.5]	2.6	1.2	5.2 [4.4-7.7]	3.4	0.0	4.4 [3.4-6.9]	2.0	0.0	7.1 [6-13]	2.6	3.5	4.9 [4.2-9]	2.6	0.0	7.4 [5.7-8.3]	2.3	3.6	13488	15 [10-29]	5.9 [4.8-11]	2.6	1.4	4.0
Lyso PC	6 [4.9-12]	13.7	0.0	5.4 [4.7-16]	6.8	0.0	5.1 [4-18]	13.8	0.0	8.3 [6.6-19]	6.4	3.3	5.1 [3.6-19]	11.7	0.0	8.1 [6.6-15]	6.7	0.0	5012	18 [12-66]	8.1 [6.5-28]	9.8	0.6	10.4
Sphingolipids	5.9 [4.6-9.6]	0.0	6.0	5.2 [4.5-17]	0.1	4.3	4.7 [3.9-6.9]	0.0	0.0	7.4 [6.6-14]	0.0	8.7	5 [4.3-13]	0.0	6.1	7.3 [6.5-8.3]	0.0	6.7	5113	68 [37-120]	6.7 [5.1-14]	0.0	5.3	5.3
Sum of hexoses	4.5 [1.5-8]	0.0	0.0	5.2 [1.1-7.4]	0.0	0.0	4.4 [1.7-9.1]	0.0	0.0	6.5 [2.3-17]	0.0	3.3	4.3 [1.2-6.9]	0.0	0.0	11 [4.4-23]	0.0	1.7	357	9.3 [7.5-16]	6.3 [3.9-13]	0.0	0.8	0.8
Total	5.2 [3.7-9.1]	17.3	5.1	5.5 [4.3-10]	16.2	3.0	5.2 [3.7-11]	11.7	3.7	7.3 [5.5-14]	16.3	6.6	5.4 [4.1-14]	14.4	4.0	7.4 [5.9-11]	5.6	8.2	64565	18 [8.2-61]	7.6 [5-27]	13.6	5.1	18.7

Supplementary Table S-7. Ratio of inter-laboratory to intra-laboratory % CV, for the 20 test materials and 189 metabolites, per metabolite class for the six participating laboratories.

	Inter/intra %CV ratio					
Metabolite class	LabA	LabB	LabC	LabD	LabE	LabF
Amino Acids	1.6	1.5	1.2	1.1	1.2	1.0
Biogenic Amines	6.9	4.9	3.7	3.7	3.8	3.0
Carnitines	1.8	1.6	1.8	1.5	1.3	1.5
Di-acyl PC (PC aa)	1.2	1.1	1.5	0.9	1.3	0.9
Alkyl-acyl PC (PC ae)	1.2	1.1	1.3	0.8	1.2	0.8
Lyso PC	1.3	1.5	1.6	1.0	1.6	1.0
Sphingolipids	1.1	1.3	1.4	0.9	1.3	0.9
Sum of hexoses	1.4	1.2	1.4	1.0	1.5	0.5
Total	1.5	1.4	1.5	1.0	1.4	1.0

Supplementary Table S-8. List of metabolites with BLD + missing>30% and/or median inter-lab %CV >20%. BLD: below the limit of detection.

27 metabolites (14%), BLD+missing>30%		12 metabolites (6%), %CV >20%	12 metabolites (6%), BLD+missing>30% and %CV >20%	4 metabolites, Inter-laboratory CV calculation not possible, BLD+missing>30%
C10:1	C4:1	ADMA	C3:1	cis-4-OH-Pro
C10:2	C5:1	alpha AAA	C5:1-DC	Dopamine
C12:1	C5-DC (C6-OH)	Asp	C9	Nitro-Tyr
C12-DC	C5-M-DC	Glu	Ac-Orn	PEA
C14	C5-OH (C3-DC-M)	SDMA	Carnosine	
C14:1-OH	C6 (C4:1-DC)	Spermidine	DOPA	
C14:2-OH	C6:1	lysoPC a C26:0	Histamine	
C16:1	C7-DC	lysoPC a C26:1	Met-SO	
C16:1-OH	C8	PC aa C24:0	Putrescine	
C16:2	lysoPC a C14:0	PC aa C36:0	Spermine	
C16:2-OH	PC aa C26:0	PC ae C30:1	PC aa C30:2	
C16-OH	PC aa C40:1	PC ae C38:1	SM C22:3	
C18:1-OH	PC ae C42:0			
C3-OH				

*Note: total DMA and sarcosine were removed from these lists. For total-DMA no MRM data was acquired for lab D and F, and this is why it appears with many overall missing values, otherwise there were no BLD and missing values for the laboratories that acquire the MRM data. Sarcosine: no MRM for lab A, B, C and E, therefore interlaboratory calculation was not possible. PC aa C30:2 and SM C22:3: no MRM for lab F.

Supplementary Table S-9. Summary of the medians of inter-laboratory %CV for the 20 test material, the serum samples and plasma samples with different anticoagulants, the high lipid content samples and the pooled QC (QCP). Data for laboratory D for QCP have been excluded.

Metabolite class	Median inter-laboratory %CV						
	20 Test Materials	Serum	EDTA Plasma	Citrate Plasma	Heparin Plasma	High lipid content (TS01)	QCP
Amino Acids	7.1	8	7.8	6.7	8.5	8.8	7.9
Biogenic Amines	26	30	26	26	24	28	20.8
Carnitines	12	10	13	12	11	14	8.4
Di-acyl PC (PC aa)	6.4	6	6.4	6.6	6.5	24	6.9
Alkyl-acyl PC (PC ae)	5.9	5.7	6.4	6.3	6.2	26	7.4
Lyso PC	8.1	7.4	9.5	6.9	9.6	13	8.6
Sphingolipids	6.5	5.4	7.9	4.5	8.6	25	4.2
Sum of hexoses	6.3	4.4	6	5.5	10.6	12.2	10.4
Total	7.6	6.9	7.9	7.2	8.2	20	7.8

Supplementary Table S-10. Estimation of overall and per laboratory accuracy and precision with reference to NIST SRM 1950.

		Lab A		Lab B		Lab C		Lab D		Lab E		Lab F		All labs	
	NIST Reference	Accur %	Prec %	Accur %	Prec %	Accur %	Prec %	Accur %	Prec %	Accur %	Prec %	Accur %	Prec %	Accur %	Prec %
Ala	300	101.3	2.3	102.9	1.4	101.0	8.0	103.9	0.7	102.2	2.9	103.7	4.2	102.5	1.2
Arg	81.4	97.1	8.2	111.5	7.9	99.9	10.6	117.0	7.1	109.0	8.9	112.4	9.4	107.8	7.2
Glu	67	105.3	4.0	108.1	2.8	109.7	4.4	110.7	4.0	100.7	5.5	111.4	6.3	107.0	3.9
Gly	245	97.6	6.9	110.8	6.8	109.7	11.1	109.3	7.1	115.4	14.0	99.2	1.6	107.0	6.6
His	72.6	89.8	6.9	100.1	5.5	94.4	7.0	103.2	7.5	104.2	4.5	99.1	5.7	98.4	5.6
Ile	55.5	104.5	5.9	114.2	4.7	123.7	6.1	121.1	6.2	138.7	6.8	110.5	5.0	118.8	10.1
Leu	100.4	96.7	6.1	115.6	4.8	115.9	6.5	124.2	4.8	136.5	10.1	106.8	8.1	115.9	11.8
Lys	140	97.6	8.9	117.6	6.5	95.2	8.9	125.7	7.4	110.7	3.6	126.4	7.3	112.2	12.1
Met	22.3	87.9	4.8	99.2	3.7	96.7	10.0	97.0	6.4	109.7	9.3	94.6	6.1	97.5	7.3
Orn	52.1	104.0	11.0	117.4	7.8	118.6	12.4	120.7	8.2	132.4	4.2	123.7	7.2	119.5	7.8
Phe	51	105.6	6.0	111.8	6.4	118.6	10.7	116.5	5.4	119.5	4.4	121.8	7.5	115.6	5.2
Pro	177	85.9	6.8	105.9	4.8	113.6	7.5	109.2	7.3	108.1	8.7	110.6	3.4	105.5	9.4
Ser	65.9	133.0	8.5	155.5	7.9	133.9	10.5	151.0	9.0	138.6	10.6	138.6	7.6	141.8	6.6
Thr	119.5	93.0	2.5	102.8	2.5	106.6	1.2	103.2	4.5	116.6	4.6	105.0	1.3	104.5	7.3
Tyr	57.3	93.6	7.8	106.4	4.8	97.6	8.7	103.6	6.3	99.1	6.1	101.8	7.6	100.4	4.5
Val	182.2	84.4	9.5	98.9	6.2	95.7	8.8	91.5	4.3	94.2	9.6	94.9	5.6	93.3	5.3
Creatinine	60	99.1	1.3	105.1	1.9	106.8	4.4	101.2	3.3	105.7	5.5	106.1	67.3	104.0	3.0

Supplementary Table S-11. Inter-batch precision %CV (plate to plate variability) for laboratory A for two citrate plasma QCs, 2 replicates each per plate, for 14 plates.

Supplementary Table S-11

Metabolite	Inter-batch CV (%) n=14	
	QC1	QC2
C0	2.4	2.6
C10	<LOD	<LOD
C10:1	7.9	<LOD
C10:2	<LOD	<LOD
C12	<LOD	6.4
C12-DC	<LOD	<LOD
C12:1	14.5	14.5
C14	8.8	7.7
C14:1	4.9	6.1
C14:1-OH	<LOD	<LOD
C14:2	5.4	8.0
C14:2-OH	<LOD	<LOD
C16	3.3	4.5
C16-OH	<LOD	<LOD
C16:1	<LOD	<LOD
C16:1-OH	<LOD	12.6
C16:2	<LOD	<LOD
C16:2-OH	<LOD	<LOD
C18	5.7	4.2
C18:1	2.7	5.2
C18:1-OH	<LOD	<LOD
C18:2	3.1	4.6
C2	2.4	2.7
C3	3.7	3.1
C3-OH	<LOD	<LOD
C3:1	<LOD	<LOD
C4	4.5	6.3
C3-DC (C4-OH)	<LOD	<LOD
C4:1	<LOD	<LOD
C5	4.6	4.9
C5-M-DC	<LOD	<LOD
C5-OH (C3-DC-M)	<LOD	<LOD
C5:1	<LOD	<LOD
C5:1-DC	<LOD	<LOD
C6 (C4:1-DC)	<LOD	<LOD
C5-DC (C6-OH)	8.4	<LOD
C6:1	<LOD	<LOD
C7-DC	<LOD	<LOD
C8	<LOD	<LOD
C9	<LOD	<LOD
Ala	3.5	4.1
Arg	5.3	6.5
Asn	6.6	5.2
Asp	22.0	16.7
Cit	6.7	5.6
Gln	3.9	5.8
Glu	5.6	4.6

Supplementary Table S-11

Metabolite	Inter-batch CV (%) n=14	
	QC1	QC2
Gly	4.3	7.0
His	3.4	3.8
Ile	3.5	6.5
Leu	4.2	5.9
Lys	6.6	7.6
Met	7.1	6.7
Orn	8.6	9.8
Phe	3.2	4.7
Pro	4.2	5.8
Ser	4.6	6.3
Thr	4.0	5.2
Trp	3.2	5.9
Tyr	5.0	4.5
Val	4.7	6.4
Ac-Orn	<LOD	<LOD
alpha-AAA	<LOD	<LOD
c4-OH-Pro	<LOD	<LOD
Carnosine	<LOD	<LOD
Creatinine	6.0	4.9
DOPA	N/A	N/A
Dopamine	N/A	N/A
Histamine	<LOD	<LOD
Kynurenine	6.6	5.5
Met-SO	<LOD	<LOD
Nitro-Tyr	<LOD	<LOD
PEA	<LOD	<LOD
Putrescine	<LOD	<LOD
Sarcosine	6.9	8.4
Serotonin	13.5	8.7
Spermidine	<LOD	<LOD
Spermine	<LOD	<LOD
t4-OH-Pro	5.9	7.6
Taurine	2.0	3.3
ADMA	11.3	11.3
SDMA	7.1	9.0
lysoPC a C14:0	3.7	<LOD
lysoPC a C16:0	3.5	3.9
lysoPC a C16:1	4.7	5.9
lysoPC a C17:0	3.9	4.2
lysoPC a C18:0	3.2	4.3
lysoPC a C18:1	3.0	3.9
lysoPC a C18:2	3.6	4.2
lysoPC a C20:3	4.5	4.5
lysoPC a C20:4	4.2	5.5
lysoPC a C24:0	9.7	12.0
lysoPC a C26:0	28.5	21.9
lysoPC a C26:1	31.2	29.2
lysoPC a C28:0	15.5	11.7
lysoPC a C28:1	14.3	13.2

Supplementary Table S-11

Metabolite	Inter-batch CV (%) n=14	
	QC1	QC2
PC aa C24:0	23.8	17.5
PC aa C26:0	<LOD	<LOD
PC aa C28:1	3.1	3.8
PC aa C30:0	3.5	3.6
PC aa C30:2	53.2	66.3
PC aa C32:0	6.2	5.6
PC aa C32:1	5.5	6.2
PC aa C32:2	6.2	7.7
PC aa C32:3	6.8	8.0
PC aa C34:1	5.2	4.5
PC aa C34:2	9.8	9.6
PC aa C34:3	8.1	8.2
PC aa C34:4	4.6	4.3
PC aa C36:0	4.3	4.4
PC aa C36:1	4.9	5.1
PC aa C36:2	7.4	7.1
PC aa C36:3	4.4	4.7
PC aa C36:4	4.7	4.5
PC aa C36:5	4.7	4.3
PC aa C36:6	5.3	6.2
PC aa C38:0	5.0	3.6
PC aa C38:1	21.6	17.3
PC aa C38:3	3.4	3.9
PC aa C38:4	3.5	3.2
PC aa C38:5	5.5	5.2
PC aa C38:6	5.7	6.5
PC aa C40:1	<LOD	<LOD
PC aa C40:2	6.2	6.6
PC aa C40:3	6.3	9.6
PC aa C40:4	2.6	2.9
PC aa C40:5	4.4	4.7
PC aa C40:6	3.8	4.5
PC aa C42:0	5.7	4.8
PC aa C42:1	3.8	6.3
PC aa C42:2	8.1	5.1
PC aa C42:4	8.4	10.3
PC aa C42:5	8.9	6.4
PC aa C42:6	6.8	3.9
PC ae C30:0	5.5	4.7
PC ae C30:1	32.3	41.1
PC ae C30:2	8.9	6.3
PC ae C32:1	8.9	9.3
PC ae C32:2	9.5	9.9
PC ae C34:0	6.4	8.0
PC ae C34:1	7.8	8.2
PC ae C34:2	5.9	6.2
PC ae C34:3	6.5	5.8
PC ae C36:0	11.6	9.0
PC ae C36:1	4.7	5.1

Supplementary Table S-11

Metabolite	Inter-batch CV (%) n=14	
	QC1	QC2
PC ae C36:2	4.6	4.7
PC ae C36:3	4.4	4.9
PC ae C36:4	7.2	7.8
PC ae C36:5	7.8	8.2
PC ae C38:0	6.8	6.5
PC ae C38:1	88.7	85.8
PC ae C38:2	9.6	10.2
PC ae C38:3	4.5	6.0
PC ae C38:4	6.6	7.1
PC ae C38:5	4.2	4.9
PC ae C38:6	4.4	4.4
PC ae C40:1	4.3	4.3
PC ae C40:2	4.5	3.6
PC ae C40:3	4.2	5.1
PC ae C40:4	6.5	6.3
PC ae C40:5	3.3	3.3
PC ae C40:6	4.0	3.4
PC ae C42:0	<LOD	<LOD
PC ae C42:1	4.9	7.1
PC ae C42:2	4.8	5.7
PC ae C42:3	6.2	4.8
PC ae C42:4	4.9	5.0
PC ae C42:5	3.2	2.8
PC ae C44:3	9.2	10.6
PC ae C44:4	7.6	7.0
PC ae C44:5	3.7	5.1
PC ae C44:6	4.6	3.7
SM (OH) C14:1	6.8	5.6
SM (OH) C16:1	4.6	4.2
SM (OH) C22:1	5.5	6.1
SM (OH) C22:2	10.9	9.0
SM (OH) C24:1	10.3	12.4
SM C16:0	6.0	6.0
SM C16:1	5.4	5.0
SM C18:0	6.5	6.2
SM C18:1	5.7	6.1
SM C20:2	17.9	7.3
SM C22:3	152.6	242.7
SM C24:0	11.2	10.8
SM C24:1	4.8	5.2
SM C26:0	14.7	13.0
SM C26:1	10.5	9.8
H1	2.8	2.3

Supplementary Table S-12. Inter-batch precision %CV (plate to plate variability) for laboratory B for two QC materials, the SRM NIST 1950 and a commercial available human serum (CQC). Also mean intra-batch precision %CV for the SRM NIST 1950. 4 replicates each per plate, for 4 plates for the SRM NIST 1950 and 2 replicates each per plate, for 4 plates for CQC.

Supplementary Table S-12

Metabolite	inter-batch CV (%)	mean intra-batch CV (%)	inter-batch CV (%)
	4 replicates/plate, 4 plates	4 replicates/plate, 4 plates	2 replicates/plate, 4 plates
	SRM NIST 1950	SRM NIST 1950	CQC
C0	2.62	6.30	1.93
C10	4.27	5.76	3.93
C10:1	9.17	6.64	5.02
C10:2	5.90	8.36	7.88
C12	1.78	7.49	2.56
C12-DC	5.35	7.92	6.42
C12:1	5.40	7.75	0.79
C14	17.43	7.29	22.87
C14:1	1.62	12.55	2.87
C14:1-OH	3.06	12.17	3.60
C14:2	1.95	8.81	2.97
C14:2-OH	7.18	11.25	10.76
C16	5.36	7.31	2.67
C16-OH	3.83	10.98	5.39
C16:1	2.42	9.01	3.54
C16:1-OH	11.59	10.08	7.18
C16:2	4.34	8.62	7.39
C16:2-OH	2.81	12.76	3.63
C18	4.91	7.53	2.97
C18:1	2.06	7.54	5.91
C18:1-OH	47.59	8.52	49.50
C18:2	4.36	8.17	5.31
C2	2.78	6.26	2.32
C3	25.19	5.68	22.22
C5-OH (C3-DC-M)	5.44	6.25	10.16
C3-OH	21.24	15.32	23.36
C3:1	80.50	8.99	86.50
C4	23.69	7.73	20.75
C3-DC (C4-OH)	22.32	4.58	15.77
C4:1	12.19	6.71	16.48
C5	2.29	6.70	5.02
C5-DC (C6-OH)	17.55	7.27	19.39
C5-M-DC	17.61	8.57	20.14
C5:1	5.28	14.16	10.12
C5:1-DC	9.28	12.67	12.21
C6 (C4:1-DC)	8.51	6.62	2.33
C6:1	7.22	11.57	9.49
C7-DC	14.58	6.01	17.81
C8	7.48	8.52	10.33
C9	7.44	6.07	11.89
Ala	2.51	4.46	4.92
Arg	4.47	6.90	5.26
Asn	2.59	4.76	7.42
Asp	5.75	9.55	3.44
Cit	3.30	4.41	4.07
Gln	1.91	6.89	6.50
Glu	2.27	4.89	5.68

Supplementary Table S-12

Metabolite	inter-batch CV (%)	mean intra-batch CV (%)	inter-batch CV (%)
	4 replicates/plate, 4 plates	4 replicates/plate, 4 plates	2 replicates/plate, 4 plates
	SRM NIST 1950	SRM NIST 1950	CQC
Gly	4.50	5.76	5.09
His	2.31	6.71	3.27
Ile	4.91	5.95	5.95
Leu	4.30	6.55	3.66
Lys	4.88	7.60	5.51
Met	11.58	8.36	12.01
Orn	3.88	6.43	6.65
Phe	4.33	5.93	4.65
Pro	3.92	6.14	6.67
Ser	2.03	6.26	3.17
Thr	1.12	4.14	4.67
Trp	2.87	5.71	4.83
Tyr	5.55	6.62	6.53
Val	3.88	6.47	5.34
Ac-Orn	10.99	9.80	16.92
ADMA	2.16	9.76	4.60
alpha-AAA	18.56	9.37	17.38
c4-OH-Pro	NA	NA	NA
Carnosine	100.56	NA	173.21
Creatinine	2.36	3.63	7.02
DOPA	13.19	6.67	14.59
Dopamine	59.07	NA	NA
Histamine	47.94	2.32	48.74
Kynurenine	4.34	6.77	5.20
Met-SO	10.14	6.99	12.13
Nitro-Tyr	NA	NA	173.21
PEA	173.21	NA	173.21
Putrescine	57.02	31.09	110.55
SDMA	32.88	11.74	14.34
Serotonin	26.18	10.31	50.00
Spermidine	4.30	4.60	4.79
Spermine	2.40	6.28	4.24
t4-OH-Pro	6.49	6.77	9.45
Taurine	3.00	5.04	4.75
total DMA	7.15	6.44	5.62
lysoPC a C14:0	1.26	6.73	1.94
lysoPC a C16:0	4.22	7.40	3.69
lysoPC a C16:1	4.33	7.33	2.69
lysoPC a C17:0	4.52	8.60	3.65
lysoPC a C18:0	4.72	7.19	3.93
lysoPC a C18:1	4.59	7.75	3.61
lysoPC a C18:2	4.65	8.27	3.25
lysoPC a C20:3	4.60	7.24	3.45
lysoPC a C20:4	6.13	8.03	3.74
lysoPC a C24:0	20.34	30.09	9.04
lysoPC a C26:0	31.91	43.84	34.67
lysoPC a C26:1	37.07	39.95	36.02
lysoPC a C28:0	17.02	29.11	16.69
lysoPC a C28:1	25.66	33.43	21.20

Supplementary Table S-12

Metabolite	inter-batch CV (%)	mean intra-batch CV (%)	inter-batch CV (%)
	4 replicates/plate, 4 plates	4 replicates/plate, 4 plates	2 replicates/plate, 4 plates
	SRM NIST 1950	SRM NIST 1950	CQC
PC aa C24:0	28.35	40.77	35.97
PC aa C26:0	29.77	31.03	33.58
PC aa C28:1	8.67	9.23	9.60
PC aa C30:0	4.16	6.07	3.75
PC aa C30:2	22.70	30.51	30.51
PC aa C32:0	9.51	7.57	9.53
PC aa C32:1	10.65	7.57	9.26
PC aa C32:2	10.32	9.96	14.23
PC aa C32:3	11.66	8.60	12.57
PC aa C34:1	8.57	7.61	7.36
PC aa C34:2	9.40	7.80	8.80
PC aa C34:3	8.98	7.90	6.82
PC aa C34:4	6.62	8.24	8.07
PC aa C36:0	7.67	9.66	5.56
PC aa C36:1	6.19	8.43	7.32
PC aa C36:2	6.85	7.86	7.86
PC aa C36:3	6.70	7.64	6.67
PC aa C36:4	7.58	8.16	8.43
PC aa C36:5	7.52	8.18	7.23
PC aa C36:6	5.53	8.89	3.89
PC aa C38:0	6.14	7.66	5.71
PC aa C38:1	16.42	32.36	19.63
PC aa C38:3	5.03	6.60	4.69
PC aa C38:4	6.17	7.87	5.22
PC aa C38:5	5.51	7.09	4.73
PC aa C38:6	5.91	7.96	6.39
PC aa C40:1	4.39	8.70	1.72
PC aa C40:2	8.88	13.00	4.93
PC aa C40:3	10.86	12.40	7.41
PC aa C40:4	4.05	8.71	5.21
PC aa C40:5	3.82	8.14	4.98
PC aa C40:6	3.70	8.34	6.33
PC aa C42:0	2.96	9.13	9.50
PC aa C42:1	9.21	13.61	6.14
PC aa C42:2	5.75	13.82	4.93
PC aa C42:4	1.25	11.41	8.30
PC aa C42:5	3.55	10.35	3.67
PC aa C42:6	5.00	8.46	5.07
PC ae C30:0	7.33	12.60	8.05
PC ae C30:1	43.83	38.48	57.77
PC ae C30:2	21.05	23.58	12.93
PC ae C32:1	12.32	8.82	11.61
PC ae C32:2	15.29	10.45	14.72
PC ae C34:0	10.30	7.11	8.56
PC ae C34:1	8.54	7.35	10.75
PC ae C34:2	9.04	8.36	9.61
PC ae C34:3	8.45	7.87	10.06
PC ae C36:0	11.01	9.87	15.96
PC ae C36:1	9.61	6.27	9.08

Supplementary Table S-12

Metabolite	inter-batch CV (%)	mean intra-batch CV (%)	inter-batch CV (%)
	4 replicates/plate, 4 plates	4 replicates/plate, 4 plates	2 replicates/plate, 4 plates
	SRM NIST 1950	SRM NIST 1950	CQC
PC ae C36:2	7.34	7.83	8.86
PC ae C36:3	7.40	8.65	8.27
PC ae C36:4	6.89	8.72	7.24
PC ae C36:5	7.43	9.09	8.61
PC ae C38:0	7.11	8.45	6.90
PC ae C38:1	19.63	27.17	8.11
PC ae C38:2	7.65	6.96	9.33
PC ae C38:3	5.98	7.58	6.69
PC ae C38:4	7.09	7.53	5.50
PC ae C38:5	5.43	8.63	4.92
PC ae C38:6	5.35	8.25	6.56
PC ae C40:1	7.45	13.52	9.29
PC ae C40:2	7.06	9.83	7.14
PC ae C40:3	10.17	10.29	5.69
PC ae C40:4	4.84	8.52	6.26
PC ae C40:5	4.58	8.95	5.65
PC ae C40:6	5.10	7.96	5.09
PC ae C42:0	2.93	7.68	3.34
PC ae C42:1	8.52	16.41	10.58
PC ae C42:2	4.04	10.03	6.10
PC ae C42:3	3.61	11.31	3.35
PC ae C42:4	2.62	9.12	4.20
PC ae C42:5	2.67	7.32	2.99
PC ae C44:3	11.01	21.22	4.86
PC ae C44:4	2.29	11.00	4.26
PC ae C44:5	1.92	8.43	3.28
PC ae C44:6	1.44	7.61	5.45
SM (OH) C14:1	3.36	6.02	1.82
SM (OH) C16:1	2.78	5.57	1.00
SM (OH) C22:1	4.49	7.85	6.08
SM (OH) C22:2	3.61	7.62	5.04
SM (OH) C24:1	7.62	9.60	13.47
SM C16:0	3.28	5.58	1.76
SM C16:1	3.28	5.73	1.18
SM C18:0	2.84	5.01	1.92
SM C18:1	2.40	5.96	2.03
SM C20:2	3.78	9.11	7.41
SM C22:3	54.26	72.58	50.33
SM C24:0	5.45	5.05	6.43
SM C24:1	4.07	6.28	7.02
SM C26:0	17.48	22.64	33.74
SM C26:1	11.01	22.93	15.83
H1	2.04	5.88	1.91

Supplementary Table S-13. Inter-batch precision %CV (plate to plate variability) and mean intra-batch precision %CV for lab C, for a commercially available reference plasma, 5 replicates each per plate, for 4 plates.

Supplementary Table S-13

metabolite	inter-batch CV (%)	mean intra-batch CV (%)
	5 replicates/plate, 4 plates	5 replicates/plate, 4 plates
C0	5.6	4.6
C10	11.8	5.9
C10:1	3.2	7.8
C10:2	7.3	10.8
C12	8.4	11.3
C12-DC	31.2	4.4
C12:1	16.1	13.0
C14	9.7	18.7
C14:1	5.1	8.8
C14:1-OH	5.1	14.2
C14:2	6.3	10.1
C14:2-OH	5.8	11.9
C16	2.9	10.5
C16-OH	6.9	10.0
C16:1	15.6	4.5
C16:1-OH	8.3	16.7
C16:2	18.1	9.7
C16:2-OH	3.0	8.1
C18	5.9	11.6
C18:1	4.2	6.0
C18:1-OH	6.9	12.4
C18:2	10.1	6.8
C2	2.3	4.7
C3	9.0	6.5
C5-OH (C3-DC-M)	28.3	12.0
C3-OH	29.1	12.7
C3:1	30.9	12.8
C4	3.2	6.9
C3-DC (C4-OH)	40.9	8.9
C4:1	28.9	9.8
C5	7.0	8.7
C5-DC (C6-OH)	27.9	10.2
C5-M-DC	21.2	6.9
C5:1	14.2	10.7
C5:1-DC	24.6	13.3
C6 (C4:1-DC)	20.0	7.8
C6:1	33.3	13.5
C7-DC	17.4	10.5
C8	12.9	5.2
C9	8.9	11.5
Ala	1.8	4.5
Arg	8.3	6.5
Asn	8.4	8.8
Asp	5.9	7.3
Cit	5.9	10.5
Gln	13.7	8.2
Glu	6.8	5.6

Supplementary Table S-13

metabolite	inter-batch CV (%)	mean intra-batch CV (%)
	5 replicates/plate, 4 plates	5 replicates/plate, 4 plates
Gly	10.0	5.3
His	6.3	5.7
Ile	6.1	5.3
Leu	7.5	5.6
Lys	12.4	8.2
Met	6.1	6.8
Orn	8.6	6.2
Phe	9.0	4.7
Pro	7.3	5.0
Ser	5.5	8.9
Thr	0.6	5.3
Trp	6.6	5.7
Tyr	6.6	5.4
Val	7.2	7.4
Ac-Orn	14.7	9.6
ADMA	15.2	13.7
alpha-AAA	49.7	20.9
c4-OH-Pro	NA	NA
Carnosine	NA	NA
Creatinine	2.5	3.4
DOPA	NA	NA
Dopamine	NA	NA
Histamine	NA	NA
Kynurenine	8.6	9.7
Met-SO	21.1	12.2
Nitro-Tyr	NA	NA
PEA	NA	NA
Putrescine	56.2	8.9
SDMA	38.7	16.7
Serotonin	46.1	7.5
Spermidine	29.4	2.9
Spermine	26.8	1.9
t4-OH-Pro	13.0	9.6
Taurine	6.4	6.6
total DMA	17.3	11.1
lysoPC a C14:0	7.2	4.3
lysoPC a C16:0	8.7	6.2
lysoPC a C16:1	7.2	7.0
lysoPC a C17:0	8.6	7.7
lysoPC a C18:0	8.6	6.1
lysoPC a C18:1	8.7	6.6
lysoPC a C18:2	8.3	6.2
lysoPC a C20:3	4.3	5.9
lysoPC a C20:4	7.5	5.3
lysoPC a C24:0	4.8	12.0
lysoPC a C26:0	22.4	17.1
lysoPC a C26:1	63.2	21.9
lysoPC a C28:0	43.4	12.2
lysoPC a C28:1	30.1	10.8

Supplementary Table S-13

metabolite	inter-batch CV (%)	mean intra-batch CV (%)
	5 replicates/plate, 4 plates	5 replicates/plate, 4 plates
PC aa C24:0	13.9	40.2
PC aa C26:0	11.8	4.1
PC aa C28:1	11.4	5.0
PC aa C30:0	10.0	5.6
PC aa C30:2	6.4	10.1
PC aa C32:0	6.9	5.7
PC aa C32:1	3.5	4.5
PC aa C32:2	3.7	7.5
PC aa C32:3	5.5	6.9
PC aa C34:1	5.2	5.0
PC aa C34:2	3.9	4.9
PC aa C34:3	5.1	4.8
PC aa C34:4	7.7	5.4
PC aa C36:0	4.3	5.1
PC aa C36:1	8.1	5.6
PC aa C36:2	4.8	4.8
PC aa C36:3	3.9	4.5
PC aa C36:4	3.8	4.8
PC aa C36:5	3.0	4.5
PC aa C36:6	7.9	8.2
PC aa C38:0	3.2	4.8
PC aa C38:1	12.3	15.9
PC aa C38:3	5.0	4.9
PC aa C38:4	3.6	4.4
PC aa C38:5	3.4	4.9
PC aa C38:6	2.9	4.6
PC aa C40:1	3.3	6.6
PC aa C40:2	5.6	7.5
PC aa C40:3	7.5	6.4
PC aa C40:4	2.7	5.0
PC aa C40:5	2.9	5.2
PC aa C40:6	2.6	4.6
PC aa C42:0	2.9	6.7
PC aa C42:1	2.5	7.2
PC aa C42:2	1.4	6.8
PC aa C42:4	5.3	9.4
PC aa C42:5	1.7	8.1
PC aa C42:6	3.2	6.1
PC ae C30:0	4.8	7.6
PC ae C30:1	10.4	19.5
PC ae C30:2	3.7	8.2
PC ae C32:1	4.9	5.3
PC ae C32:2	8.1	5.8
PC ae C34:0	9.3	5.2
PC ae C34:1	4.9	5.1
PC ae C34:2	2.9	4.6
PC ae C34:3	5.6	4.9
PC ae C36:0	9.8	6.2
PC ae C36:1	4.2	4.4

Supplementary Table S-13

metabolite	inter-batch CV (%)	mean intra-batch CV (%)
	5 replicates/plate, 4 plates	5 replicates/plate, 4 plates
PC ae C36:2	4.1	4.9
PC ae C36:3	6.5	4.9
PC ae C36:4	2.8	5.2
PC ae C36:5	3.2	4.7
PC ae C38:0	5.5	5.0
PC ae C38:1	3.0	7.8
PC ae C38:2	0.8	5.1
PC ae C38:3	1.1	4.7
PC ae C38:4	3.4	5.1
PC ae C38:5	2.5	4.9
PC ae C38:6	2.8	4.3
PC ae C40:1	4.6	4.6
PC ae C40:2	5.5	5.1
PC ae C40:3	1.9	4.9
PC ae C40:4	1.3	4.3
PC ae C40:5	1.5	4.7
PC ae C40:6	2.4	5.0
PC ae C42:0	1.3	6.4
PC ae C42:1	6.2	6.4
PC ae C42:2	3.8	5.7
PC ae C42:3	4.5	7.2
PC ae C42:4	1.8	6.2
PC ae C42:5	2.2	4.2
PC ae C44:3	9.3	11.7
PC ae C44:4	3.7	7.4
PC ae C44:5	2.6	5.6
PC ae C44:6	2.6	5.5
SM (OH) C14:1	0.6	6.0
SM (OH) C16:1	0.8	7.1
SM (OH) C22:1	1.3	4.8
SM (OH) C22:2	1.5	5.9
SM (OH) C24:1	6.3	7.8
SM C16:0	0.7	6.2
SM C16:1	1.8	5.3
SM C18:0	0.9	6.3
SM C18:1	0.8	5.9
SM C20:2	14.0	9.5
SM C22:3	19.3	10.4
SM C24:0	1.8	5.9
SM C24:1	1.9	5.7
SM C26:0	4.6	16.3
SM C26:1	3.4	8.3
H1	2.8	4.5

Supplementary Table S-14. Baseline analytical performance of the kit and performance limitations in normal human plasma, as provided by the kit manufacturer. CV calculated with 6 replicates of QC samples per plate, for 4 plates.

Supplementary Table S-14

Analytical Performance of the Absolute/DQ® p180 Kit – Classification of the Analytes

LC						
Metabolite	Class	Category	Performance limitations human normal plasma	Calibration points	Calibration range [µM]	QC
Ala	aminoacids	1	TQ-S micro: LLOQ = Cal2	7	20 - 1600	X
Arg	aminoacids	1		7	5 - 400	X
Asn	aminoacids	1		7	5 - 400	X
Asp	aminoacids	2	CV = 15% - 30%	7	5 - 400	X
Cit	aminoacids	1		7	5 - 400	X
Gln	aminoacids	1		7	20 - 1600	X
Glu	aminoacids	1		7	10 - 800	X
Gly	aminoacids	1		7	25 - 2000	X
His	aminoacids	1		7	5 - 400	X
Ile	aminoacids	1		7	5 - 400	X
Leu	aminoacids	1	LLOQ = Cal3	7	5 - 400	X
Lys	aminoacids	1	potential derivatization limitations for ISTD	7	10 - 800	X
Met	aminoacids	1		7	5 - 400	X
Orn	aminoacids	1	potential derivatization limitations for ISTD	7	5 - 400	X
Phe	aminoacids	1		7	5 - 400	X
Pro	aminoacids	1		7	10 - 800	X
Ser	aminoacids	1		7	5 - 400	X
Thr	aminoacids	1		7	5 - 400	X
Trp	aminoacids	1		7	5 - 400	X
Tyr	aminoacids	1		7	5 - 400	X
Val	aminoacids	1		7	10 - 800	X
Ac-Orn	biogenic amines	2	LLOQ = Cal2; CV = 15% - 30%	7	0.5 - 40	X
ADMA	biogenic amines	2	CV = 15% - 30%	7	0.25 - 20	X
alpha-AAA	biogenic amines	2	< LLOQ; CV = 15% - 30%; TQ-S micro: LLOQ = Cal2	7	1 - 80	X
Carnosine	biogenic amines	1	< LLOQ	7	0.5 - 40	X
Creatinine (SCIEX)	biogenic amines	1		7	10 - 800	x
Creatinine (Waters)	biogenic amines	2	CV = 15% - 30%; TQ-S micro: LLOQ = Cal2	7	0.5 - 40	X
DOPA	biogenic amines	2	< LLOQ; CV = 15% - 30%	7	0.5 - 40	X
Dopamine	biogenic amines	2	< LLOQ; CV = 15% - 30%	7	1 - 80	X
Histamine	biogenic amines	2	< LLOQ; CV = 15% - 30%	7	1 - 80	X
Kynurenine	biogenic amines	2	CV = 15% - 30%	7	1 - 80	X
Met-SO	biogenic amines	2	LLOQ = Cal3; CV = 15% - 30% (sample stability)	7	1 - 80	X
Nitro-Tyr	biogenic amines	2	< LLOQ; CV = 15% - 30%	7	1 - 80	X
cis-4-OH-Pro	biogenic amines	1	< LLOQ	7	1 - 80	X
trans-4-OH-Pro	biogenic amines	5	not validated yet	7	1 - 80	
PEA	biogenic amines	2	< LLOQ; CV = 15% - 30%; Waters: LLOQ = Cal3	7	0.1 - 8	X
Putrescine	biogenic amines	1	SCIEX 5500: LLOQ = Cal3; Waters: LLOQ = Cal2	7	0.1 - 8	X
Sarcosine	biogenic amines	1	only analyzed by UHPLC method; autosampler stability < 24 h	7	1 - 80	X
SDMA (HPLC)	biogenic amines	2	< LLOQ; CV = 15% - 30%	7	1 - 80	X
SDMA (UHPLC)	biogenic amines	1		7	0.1 - 8	X
Serotonin	biogenic amines	2	< LLOQ; CV = 15% - 30%	7	0.1 - 8	X
Spermidine	biogenic amines	1	potential derivatization limitations for analyte and ISTD	7	0.25 - 20	X
Spermine	biogenic amines	1	Waters: LLOQ = Cal3; potential derivatization limitations for analyte and ISTD	7	0.25 - 20	X
Taurine	biogenic amines	1		7	2.5 - 200	X
total DMA	biogenic amines	1	only analyzed by HPLC method	7	1.25 - 100	X

FIA						
Metabolite	Class	Category	Performance limitations human normal plasma	Calibration points	Quant range [µM]	QC
C0	acylcarnitines	3		1	5 - 120	X
C10	acylcarnitines	3	< LLOQ	1	0.3 - 6	X
C10:1	acylcarnitines	4	< LOD	relative quant		
C10:2	acylcarnitines	4	< LOD	relative quant		
C12	acylcarnitines	3	< LLOQ	1	0.4 - 12	X
C12:1	acylcarnitines	4	< LOD	relative quant		
C12-DC	acylcarnitines	4	< LOD	relative quant		
C14	acylcarnitines	3	< LOD	1	0.4 - 6	X
C14:1	acylcarnitines	4		relative quant		
C14:1-OH	acylcarnitines	4	< LOD	relative quant		
C14:2	acylcarnitines	4		relative quant		
C14:2-OH	acylcarnitines	4	< LOD	relative quant		
C16	acylcarnitines	3	< LLOQ	1	0.4 - 12	X
C16:1	acylcarnitines	4	< LOD	relative quant		
C16:1-OH	acylcarnitines	4	< LOD	relative quant		
C16:2	acylcarnitines	4	< LOD	relative quant		
C16:2-OH	acylcarnitines	4	< LOD	relative quant		
C16-OH	acylcarnitines	4	< LOD	relative quant		
C18	acylcarnitines	3	< LLOQ	1	0.4 - 6	X
C18:1	acylcarnitines	4		relative quant		
C18:1-OH	acylcarnitines	4	< LOD	relative quant		
C18:2	acylcarnitines	4		relative quant		
C2	acylcarnitines	3		1	0.4 - 35	X
C3	acylcarnitines	3		1	0.4 - 15	X
C3:1	acylcarnitines	4	< LOD	relative quant		
C3-DC (C4-OH)	acylcarnitines	4	< LOD	relative quant		
C3-OH	acylcarnitines	4	< LOD	relative quant		

Supplementary Table S-14

Analytical Performance of the Absolute/DQ® p180 Kit – Classification of the Analytes

C4	acylcarnitines	3	< LLOQ	1	0.4 - 12	X
C4:1	acylcarnitines	4	< LOD	relative quant		
C5	acylcarnitines	3	< LLOQ	1	0.4 - 12	X
C5:1	acylcarnitines	4	< LOD	relative quant		
C5:1-DC	acylcarnitines	4	< LOD	relative quant		
C5-DC (C6-OH)	acylcarnitines	4	< LOD	relative quant		
C5-M-DC	acylcarnitines	4	< LOD	relative quant		
C5-OH (C3-DC-M)	acylcarnitines	4	< LOD	relative quant		
C6 (C4:1-DC)	acylcarnitines	3	< LOD	1	0.2 - 6	X
C6:1	acylcarnitines	4	< LOD	relative quant		
C7-DC	acylcarnitines	4	< LOD	relative quant		
C8	acylcarnitines	3	< LOD	1	0.2 - 8	X
C9	acylcarnitines	4	< LOD	relative quant		
lysoPC a C14:0	glycerophospholipids	4	< LOD	relative quant		
lysoPC a C16:0	glycerophospholipids	4		relative quant		
lysoPC a C16:1	glycerophospholipids	4		relative quant		
lysoPC a C17:0	glycerophospholipids	4		relative quant		
lysoPC a C18:0	glycerophospholipids	4		relative quant		X
lysoPC a C18:1	glycerophospholipids	4		relative quant		
lysoPC a C18:2	glycerophospholipids	4		relative quant		
lysoPC a C20:3	glycerophospholipids	4		relative quant		
lysoPC a C20:4	glycerophospholipids	4		relative quant		
lysoPC a C24:0	glycerophospholipids	4	< LOD	relative quant		
lysoPC a C26:0	glycerophospholipids	4	< LOD	relative quant		
lysoPC a C26:1	glycerophospholipids	4	< LOD	relative quant		
lysoPC a C28:0	glycerophospholipids	4	< LOD	relative quant		
lysoPC a C28:1	glycerophospholipids	4		relative quant		
PC aa C24:0	glycerophospholipids	4	< LOD	relative quant		X
PC aa C26:0	glycerophospholipids	4	< LOD	relative quant		
PC aa C28:1	glycerophospholipids	4		relative quant		
PC aa C30:0	glycerophospholipids	4		relative quant		
PC aa C30:2	glycerophospholipids	4	CV = 15% - 45% (strong isotope correction); not analyzed with Waters	relative quant		
PC aa C32:0	glycerophospholipids	4		relative quant		
PC aa C32:1	glycerophospholipids	4		relative quant		
PC aa C32:2	glycerophospholipids	4	CV = 15% - 45% (strong isotope correction)	relative quant		
PC aa C32:3	glycerophospholipids	4		relative quant		
PC aa C34:1	glycerophospholipids	4		relative quant		X
PC aa C34:2	glycerophospholipids	4		relative quant		
PC aa C34:3	glycerophospholipids	4		relative quant		
PC aa C34:4	glycerophospholipids	4		relative quant		
PC aa C36:0	glycerophospholipids	4		relative quant		
PC aa C36:1	glycerophospholipids	4		relative quant		
PC aa C36:2	glycerophospholipids	4		relative quant		
PC aa C36:3	glycerophospholipids	4		relative quant		
PC aa C36:4	glycerophospholipids	4		relative quant		
PC aa C36:5	glycerophospholipids	4		relative quant		
PC aa C36:6	glycerophospholipids	4		relative quant		
PC aa C38:0	glycerophospholipids	4		relative quant		
PC aa C38:1	glycerophospholipids	4	CV = 15% - 45% (strong isotope correction); not analyzed with Waters	relative quant		
PC aa C38:3	glycerophospholipids	4		relative quant		
PC aa C38:4	glycerophospholipids	4		relative quant		
PC aa C38:5	glycerophospholipids	4		relative quant		
PC aa C38:6	glycerophospholipids	4		relative quant		
PC aa C40:1	glycerophospholipids	4	< LOD	relative quant		
PC aa C40:2	glycerophospholipids	4		relative quant		
PC aa C40:3	glycerophospholipids	4		relative quant		
PC aa C40:4	glycerophospholipids	4		relative quant		
PC aa C40:5	glycerophospholipids	4		relative quant		
PC aa C40:6	glycerophospholipids	4		relative quant		
PC aa C42:0	glycerophospholipids	4		relative quant		
PC aa C42:1	glycerophospholipids	4		relative quant		
PC aa C42:2	glycerophospholipids	4		relative quant		
PC aa C42:4	glycerophospholipids	4		relative quant		
PC aa C42:5	glycerophospholipids	4		relative quant		
PC aa C42:6	glycerophospholipids	4		relative quant		
PC ae C30:0	glycerophospholipids	4		relative quant		
PC ae C30:1	glycerophospholipids	4	CV = 15% - 45% (strong isotope correction)	relative quant		
PC ae C30:2	glycerophospholipids	4		relative quant		
PC ae C32:1	glycerophospholipids	4		relative quant		
PC ae C32:2	glycerophospholipids	4		relative quant		
PC ae C34:0	glycerophospholipids	4		relative quant		
PC ae C34:1	glycerophospholipids	4		relative quant		
PC ae C34:2	glycerophospholipids	4		relative quant		
PC ae C34:3	glycerophospholipids	4		relative quant		
PC ae C36:0	glycerophospholipids	4		relative quant		
PC ae C36:1	glycerophospholipids	4		relative quant		
PC ae C36:2	glycerophospholipids	4		relative quant		
PC ae C36:3	glycerophospholipids	4		relative quant		
PC ae C36:4	glycerophospholipids	4		relative quant		

Supplementary Table S-14

Analytical Performance of the Absolute/DQ® p180 Kit – Classification of the Analytes

PC ae C36:5	glycerophospholipids	4		relative quant		
PC ae C38:0	glycerophospholipids	4		relative quant		
PC ae C38:1	glycerophospholipids	4	CV = 15% - 45% (strong isotope correction)	relative quant		
PC ae C38:2	glycerophospholipids	4	CV = 15% - 45% (strong isotope correction)	relative quant		
PC ae C38:3	glycerophospholipids	4		relative quant		
PC ae C38:4	glycerophospholipids	4		relative quant		
PC ae C38:5	glycerophospholipids	4		relative quant		
PC ae C38:6	glycerophospholipids	4		relative quant		
PC ae C40:1	glycerophospholipids	4		relative quant		
PC ae C40:2	glycerophospholipids	4		relative quant		
PC ae C40:3	glycerophospholipids	4		relative quant		
PC ae C40:4	glycerophospholipids	4		relative quant		
PC ae C40:5	glycerophospholipids	4		relative quant		
PC ae C40:6	glycerophospholipids	4		relative quant		
PC ae C42:0	glycerophospholipids	4	< LOD	relative quant		
PC ae C42:1	glycerophospholipids	4		relative quant		
PC ae C42:2	glycerophospholipids	4		relative quant		
PC ae C42:3	glycerophospholipids	4		relative quant		
PC ae C42:4	glycerophospholipids	4		relative quant		
PC ae C42:5	glycerophospholipids	4		relative quant		
PC ae C44:3	glycerophospholipids	4		relative quant		
PC ae C44:4	glycerophospholipids	4		relative quant		
PC ae C44:5	glycerophospholipids	4		relative quant		
PC ae C44:6	glycerophospholipids	4		relative quant		
SM (OH) C14:1	sphingolipids	4		relative quant		
SM (OH) C16:1	sphingolipids	4		relative quant		
SM (OH) C22:1	sphingolipids	4		relative quant		
SM (OH) C22:2	sphingolipids	4		relative quant		
SM (OH) C24:1	sphingolipids	4	CV = 15% - 45% (strong isotope correction)	relative quant		
SM C16:0	sphingolipids	4		relative quant		
SM C16:1	sphingolipids	4		relative quant		
SM C18:0	sphingolipids	4		relative quant		X
SM C18:1	sphingolipids	4		relative quant		
SM C20:2	sphingolipids	4	CV = 15% - 45% (strong isotope correction)	relative quant		
SM C22:3	sphingolipids	4	CV = 15% - 45% (strong isotope correction); not analyzed on Waters	relative quant		
SM C24:0	sphingolipids	4		relative quant		
SM C24:1	sphingolipids	4		relative quant		
SM C26:0	sphingolipids	4	CV = 15% - 45% (strong isotope correction)	relative quant		
SM C26:1	sphingolipids	4	CV = 15% - 45% (strong isotope correction)	relative quant		
H1	sugars	3	Low ionization efficiency on Waters TQ-S	1	200 - 30000	X

Category types:	1	LC-MS, quantitative (CV < 15%)
	2	LC-MS, quantitative with restrictions (CV = 15% - 30%)
	3	FIA-MS, quantitative, fixed internal 1-point calibration, LODs are instrument-dependent and calculated individually
	4	FIA-MS, relative quantitative (verification of accuracy not possible), LODs are instrument-dependent and calculated individually
	5	not validated yet (new analyte)

CV = coefficient of variation

Supplementary Table S-15. Limits of detection (LODs) for each metabolite calculated by each of the participating laboratory.

Supplementary Table S-15			LOD (calc.) [μM]					
Metabolite	Metabolite class		Lab A	Lab B	Lab C	Lab D	Lab E	Lab F
C0	acylcarnitines	FIA	3.28	2.8	1.89	3.31	2.16	2.16
C10	acylcarnitines	FIA	0.441	0.138	0.138	0.21	0.141	0.095
C10:1	acylcarnitines	FIA	0.306	0.122	0.054	0.105	0.356	0.272
C10:2	acylcarnitines	FIA	0.113	0.126	0.041	0.074	0.052	0.044
C12	acylcarnitines	FIA	0.112	0.08	0.072	0.122	0.066	0.068
C12-DC	acylcarnitines	FIA	0.998	0.813	0.909	0.676	0.279	0.109
C12:1	acylcarnitines	FIA	0.271	0.112	0.067	0.114	0.286	0.283
C14	acylcarnitines	FIA	0.173	0.047	0.018	0.121	0.039	0.037
C14:1	acylcarnitines	FIA	0.052	0.016	0.005	0.051	0.011	0.002
C14:1-OH	acylcarnitines	FIA	0.099	0.024	0.013	0.062	0.013	0.008
C14:2	acylcarnitines	FIA	0.031	0.017	0.009	0.037	0.012	0.006
C14:2-OH	acylcarnitines	FIA	0.053	0.019	0.016	0.045	0.01	0.002
C16	acylcarnitines	FIA	0.063	0.023	0.014	0.07	0.013	0.009
C16-OH	acylcarnitines	FIA	0.082	0.042	0.016	0.042	0.013	0.003
C16:1	acylcarnitines	FIA	0.063	0.043	0.037	0.07	0.031	0.016
C16:1-OH	acylcarnitines	FIA	0.066	0.021	0.011	0.071	0.015	0.013
C16:2	acylcarnitines	FIA	0.039	0.029	0.01	0.045	0.011	0.005
C16:2-OH	acylcarnitines	FIA	0.056	0.069	0.017	0.086	0.026	0.009
C18	acylcarnitines	FIA	0.045	0.026	0.013	0.058	0.011	0.017
C18:1	acylcarnitines	FIA	0.045	0.059	0.028	0.109	0.031	0.048
C18:1-OH	acylcarnitines	FIA	0.076	0.042	0.026	0.051	0.015	0.006
C18:2	acylcarnitines	FIA	0.027	0.016	0.008	0.035	0.008	0.006
C2	acylcarnitines	FIA	0.126	0.124	0.113	0.178	0.105	0.125
C3	acylcarnitines	FIA	0.082	0.051	0.083	0.119	0.056	0.004
C3:1	acylcarnitines	FIA	0.079	0.203	0.031	0.047	0.027	0.006
C3-DC (C4-OH)	acylcarnitines	FIA	0.064	0.045	0.109	0.091	0.028	0.019
C3-DC-M (C5-OH)	acylcarnitines	FIA	0.08	0.125	0.095	0.201	0.03	0.009
C3-OH	acylcarnitines	FIA	0.178	0.073	0.103	0.095	0.024	0.01
C4	acylcarnitines	FIA	0.091	0.034	0.074	0.075	0.056	0.048
C4:1	acylcarnitines	FIA	0.071	0.027	0.086	0.062	0.037	0.009
C5	acylcarnitines	FIA	0.083	0.046	0.102	0.105	0.042	0.008
C5:1	acylcarnitines	FIA	0.098	0.048	0.097	0.106	0.049	0.01
C5:1-DC	acylcarnitines	FIA	0.063	0.083	0.049	0.044	0.03	0.011
C5-DC (C6-OH)	acylcarnitines	FIA	0.059	0.023	0.047	0.047	0.035	0.004
C5-M-DC	acylcarnitines	FIA	0.062	0.08	0.141	0.289	0.059	0.133
C6:1	acylcarnitines	FIA	0.106	0.038	0.067	0.106	0.036	0.015
C6 (C4:1-DC)	acylcarnitines	FIA	0.295	0.038	0.098	0.091	0.067	0.042
C7-DC	acylcarnitines	FIA	0.065	0.037	0.04	0.013	0.037	0.003
C8	acylcarnitines	FIA	0.5	0.183	0.142	0.067	0.162	0.081
C9	acylcarnitines	FIA	0.213	0.177	0.034	0.063	0.029	0.007
Ala	aminoacids	LC	1.45	2.49	0	0.924	0	0
Arg	aminoacids	LC	0.139	0	0	1.46	1.81	0.822
Asn	aminoacids	LC	0	0	0	0	0	0
Asp	aminoacids	LC	0	2.25	0	6.15	0	0
Cit	aminoacids	LC	1.72	0	0	2.19	0	0
Gln	aminoacids	LC	0	0	0	10.6	0	0
Glu	aminoacids	LC	0	0	0	0	0	0
Gly	aminoacids	LC	3.29	0	0	9.96	5.07	0

Supplementary Table S-15			LOD (calc.) [μM]					
Metabolite	Metabolite class		Lab A	Lab B	Lab C	Lab D	Lab E	Lab F
His	aminoacids	LC	1.17	0.483	0	1.87	0	0
Ile	aminoacids	LC	0	0	0	1.82	0	0
Leu	aminoacids	LC	0	2.2	0	3.6	4.86	0
Lys	aminoacids	LC	5.83	2.39	0	0	4.35	0
Met	aminoacids	LC	2.76	2.39	0	1.77	0	1.85
Orn	aminoacids	LC	1.88	0.28	0	0	0	0
Phe	aminoacids	LC	0	0	0	0.161	0	0
Pro	aminoacids	LC	6.27	0	0	3.27	0	0
Ser	aminoacids	LC	0.738	0	0	3.06	3.33	0.995
Thr	aminoacids	LC	0.643	0.498	0	0	0	0
Trp	aminoacids	LC	2.96	0.95	0	0.885	1.42	2.13
Tyr	aminoacids	LC	1.11	0.7	0	2.02	2.21	0
Val	aminoacids	LC	4.8	0	0	2.13	0	0
Ac-Orn	biogenic amines	LC	0	0.261	0	0.903	0.438	1.01
ADMA	biogenic amines	LC	0	0.125	0	0	0	0
alpha-AAA	biogenic amines	LC	0	0	0	1.63	0	0
c4-OH-Pro	biogenic amines	LC	0	0	0	0.321	0.744	1.37
Carnosine	biogenic amines	LC	0	0.024	0	1.85	0	0
Creatinine	biogenic amines	LC	0	0	0	0	0.771	0
DOPA	biogenic amines	LC	0	0.995	0	0.567	0	0.793
Dopamine	biogenic amines	LC	0	0	0	0.296	0	2.78
Histamine	biogenic amines	LC	1.09	0.792	0	0	0.525	0
Kynurenine	biogenic amines	LC	0.219	0.06	0	0	0.396	0
Met-SO	biogenic amines	LC	0	0.259	0	2.23	0	0
Nitro-Tyr	biogenic amines	LC	0	1.06	0	5.22	0	1.02
PEA	biogenic amines	LC	0	0	0	0.474	0	0
Putrescine	biogenic amines	LC	0.068	0.097	0	0	0	0
Sarcosine	biogenic amines	LC	0	0	0	0	0	0
SDMA	biogenic amines	LC	0	0	0	0	0	0
Serotonin	biogenic amines	LC	0	0.014	0	0	0	0
Spermidine	biogenic amines	LC	0	0.218	0	0	0.069	0
Spermine	biogenic amines	LC	0	0.457	0	0	0	0
t4-OH-Pro	biogenic amines	LC	0	0.242	0	0.177	0	0
Taurine	biogenic amines	LC	0.436	0.679	0	2.58	0	0
total DMA	biogenic amines	LC	0	0.082	0	0	0	0
lysoPC a C14:0	glycerophospholipids	FIA	9.98	9.43	7.77	5.4	4.82	8.17
lysoPC a C16:0	glycerophospholipids	FIA	0.262	0.122	0.075	0.342	0.096	0.228
lysoPC a C16:1	glycerophospholipids	FIA	0.079	0.099	0.073	0.053	0.08	0.053
lysoPC a C17:0	glycerophospholipids	FIA	0.035	0.069	0.022	0.045	0.031	0.029
lysoPC a C18:0	glycerophospholipids	FIA	0.361	0.255	0.319	0.402	0.236	0.303
lysoPC a C18:1	glycerophospholipids	FIA	0.088	0.079	0.082	0.083	0.037	0.049
lysoPC a C18:2	glycerophospholipids	FIA	0.079	0.061	0.095	0.118	0.047	0.083
lysoPC a C20:3	glycerophospholipids	FIA	0.204	0.105	0.102	0.204	0.231	0.343
lysoPC a C20:4	glycerophospholipids	FIA	0.033	0.04	0.023	0.061	0.016	0.014
lysoPC a C24:0	glycerophospholipids	FIA	0.061	0.062	0.227	0.096	0.203	0.076
lysoPC a C26:0	glycerophospholipids	FIA	0.09	0.095	0.097	0.11	0.025	0.102
lysoPC a C26:1	glycerophospholipids	FIA	0.031	0.049	0.046	0.04	0.031	0.02
lysoPC a C28:0	glycerophospholipids	FIA	0.213	0.145	0.245	0.256	0.194	0.163

Supplementary Table S-15			LOD (calc.) [μM]					
Metabolite	Metabolite class		Lab A	Lab B	Lab C	Lab D	Lab E	Lab F
lysoPC a C28:1	glycerophospholipids	FIA	0.015	0.035	0.037	0.049	0.007	0.023
PC aa C24:0	glycerophospholipids	FIA	0.054	0.074	0.067	0.075	0.121	0.06
PC aa C26:0	glycerophospholipids	FIA	1.21	1.23	1.19	1.19	1.09	1.15
PC aa C28:1	glycerophospholipids	FIA	0.026	0.027	0.012	0.145	0.021	0.033
PC aa C30:0	glycerophospholipids	FIA	0.142	0.137	0.134	0.158	0.134	0.205
PC aa C30:2	glycerophospholipids	FIA	0.004	0.002	0.004	0	0	0
PC aa C32:0	glycerophospholipids	FIA	0.036	0.043	0.034	0.089	0.017	0.038
PC aa C32:1	glycerophospholipids	FIA	0.029	0.033	0.004	0.025	0.009	0.019
PC aa C32:2	glycerophospholipids	FIA	0.01	0.013	0.004	0.03	0.002	0
PC aa C32:3	glycerophospholipids	FIA	0.004	0.025	0.005	0.005	0.002	0.018
PC aa C34:1	glycerophospholipids	FIA	0.166	0.071	0.174	0.294	0.027	0.051
PC aa C34:2	glycerophospholipids	FIA	0.279	0.099	0.231	0.436	0.032	0.047
PC aa C34:3	glycerophospholipids	FIA	0.082	0.159	0.013	0.066	0.005	0.009
PC aa C34:4	glycerophospholipids	FIA	0.007	0.033	0.008	0.017	0.004	0.009
PC aa C36:0	glycerophospholipids	FIA	0.328	0.368	0.297	0.155	0.126	0.601
PC aa C36:1	glycerophospholipids	FIA	0.047	0.027	0.067	0.126	0.009	0.028
PC aa C36:2	glycerophospholipids	FIA	0.155	0.076	0.183	0.269	0.015	0.044
PC aa C36:3	glycerophospholipids	FIA	0.079	0.017	0.071	0.105	0.006	0.021
PC aa C36:4	glycerophospholipids	FIA	0.111	0.077	0.091	0.138	0.006	0.025
PC aa C36:5	glycerophospholipids	FIA	0.043	0.033	0.011	0.052	0.008	0.012
PC aa C36:6	glycerophospholipids	FIA	0.01	0.027	0.001	0.013	0.003	0.007
PC aa C38:0	glycerophospholipids	FIA	0.041	0.063	0.042	0.052	0.03	0.052
PC aa C38:1	glycerophospholipids	FIA	0.024	0.01	0.037	0.016	0.01	0
PC aa C38:3	glycerophospholipids	FIA	0.04	0.045	0.06	0.052	0.001	0.021
PC aa C38:4	glycerophospholipids	FIA	0.042	0.046	0.069	0.082	0.008	0.017
PC aa C38:5	glycerophospholipids	FIA	0.031	0.028	0.025	0.05	0.005	0.016
PC aa C38:6	glycerophospholipids	FIA	0.038	0.038	0.026	0.062	0	0.014
PC aa C40:1	glycerophospholipids	FIA	0.293	0.392	0.326	0.766	0.364	0.375
PC aa C40:2	glycerophospholipids	FIA	0.01	0.033	0.009	0.081	0.024	0.014
PC aa C40:3	glycerophospholipids	FIA	0.004	0.007	0.003	0.012	0.01	0.006
PC aa C40:4	glycerophospholipids	FIA	0.013	0.021	0.01	0.012	0.011	0.014
PC aa C40:5	glycerophospholipids	FIA	0	0	0	0.003	0	0
PC aa C40:6	glycerophospholipids	FIA	0.275	0.306	0.267	0.242	0.186	0.293
PC aa C42:0	glycerophospholipids	FIA	0.039	0.07	0.028	0.076	0.066	0.064
PC aa C42:1	glycerophospholipids	FIA	0.01	0.012	0.004	0.019	0.022	0.01
PC aa C42:2	glycerophospholipids	FIA	0.065	0.085	0.093	0.072	0.086	0.08
PC aa C42:4	glycerophospholipids	FIA	0.011	0.051	0.009	0.02	0.003	0.014
PC aa C42:5	glycerophospholipids	FIA	0.012	0.014	0.007	0.003	0.004	0.012
PC aa C42:6	glycerophospholipids	FIA	0.2	0.229	0.198	0.614	0.249	0.228
PC ae C30:0	glycerophospholipids	FIA	0.116	0.106	0.107	0.259	0.156	0.139
PC ae C30:1	glycerophospholipids	FIA	0.01	0	0	0.017	0.018	0
PC ae C30:2	glycerophospholipids	FIA	0.004	0.008	0.048	0.006	0.061	0.009
PC ae C32:1	glycerophospholipids	FIA	0.017	0	0	0.029	0.009	0.01
PC ae C32:2	glycerophospholipids	FIA	0.04	0.273	0.13	0.029	0.018	0.009
PC ae C34:0	glycerophospholipids	FIA	0.02	0.036	0.017	0.033	0.011	0.018
PC ae C34:1	glycerophospholipids	FIA	0.021	0.04	0.02	0.035	0.015	0.015
PC ae C34:2	glycerophospholipids	FIA	0.022	0.01	0.007	0.025	0.002	0.008
PC ae C34:3	glycerophospholipids	FIA	0.024	0.03	0.01	0.076	0.002	0.013

Supplementary Table S-15			LOD (calc.) [μM]					
Metabolite	Metabolite class		Lab A	Lab B	Lab C	Lab D	Lab E	Lab F
PC ae C36:0	glycerophospholipids	FIA	0.098	0.118	0.082	0.125	0.069	0.089
PC ae C36:1	glycerophospholipids	FIA	0.055	0.344	0.225	0.155	0.037	0.044
PC ae C36:2	glycerophospholipids	FIA	0.047	0.089	0.058	0.084	0.019	0.034
PC ae C36:3	glycerophospholipids	FIA	0.01	0.04	0.009	0.026	0.004	0.003
PC ae C36:4	glycerophospholipids	FIA	0.029	0.074	0.012	0.05	0.003	0.068
PC ae C36:5	glycerophospholipids	FIA	0.021	0.042	0.014	0.04	0.002	0.019
PC ae C38:0	glycerophospholipids	FIA	0.18	0.206	0.161	0.167	0.13	0.153
PC ae C38:1	glycerophospholipids	FIA	0.014	0.012	0.008	0.024	0.004	0.028
PC ae C38:2	glycerophospholipids	FIA	0.006	0.026	0.015	0.039	0.003	0.012
PC ae C38:3	glycerophospholipids	FIA	0.01	0.014	0.016	0.038	0.003	0.006
PC ae C38:4	glycerophospholipids	FIA	0.015	0.097	0.01	0.034	0.011	0.03
PC ae C38:5	glycerophospholipids	FIA	0.027	0.096	0.012	0.043	0.004	0.013
PC ae C38:6	glycerophospholipids	FIA	0.011	0.032	0.005	0.026	0.011	0.004
PC ae C40:1	glycerophospholipids	FIA	0.013	0.075	0.02	0.025	0.012	0.015
PC ae C40:2	glycerophospholipids	FIA	0.014	0.022	0.009	0.013	0	0.016
PC ae C40:3	glycerophospholipids	FIA	0.008	0.026	0.001	0.022	0	0.014
PC ae C40:4	glycerophospholipids	FIA	0.051	0.048	0.062	0.068	0.077	0.071
PC ae C40:5	glycerophospholipids	FIA	0.005	0.032	0.005	0.021	0.001	0.011
PC ae C40:6	glycerophospholipids	FIA	0.05	0.039	0.011	0.025	0.007	0.009
PC ae C42:0	glycerophospholipids	FIA	0.637	0.606	0.577	1.57	1.56	0.756
PC ae C42:1	glycerophospholipids	FIA	0.096	0.383	0.214	0.174	0.164	0.067
PC ae C42:2	glycerophospholipids	FIA	0.009	0.024	0.013	0.023	0.014	0.012
PC ae C42:3	glycerophospholipids	FIA	0.014	0.022	0.005	0.019	0.004	0.008
PC ae C42:4	glycerophospholipids	FIA	0	0	0	0	0	0
PC ae C42:5	glycerophospholipids	FIA	0.67	0.757	0.754	0.641	0.75	1
PC ae C44:3	glycerophospholipids	FIA	0.03	0.039	0.008	0.041	0.037	0.043
PC ae C44:4	glycerophospholipids	FIA	0.085	0.08	0.096	0.086	0.139	0.128
PC ae C44:5	glycerophospholipids	FIA	0.092	0.107	0.087	0.136	0.057	0.102
PC ae C44:6	glycerophospholipids	FIA	0.026	0.037	0.032	0.088	0.058	0.025
SM (OH) C14:1	sphingolipids	FIA	0.032	0.178	0.368	0.028	0.008	0.154
SM (OH) C16:1	sphingolipids	FIA	0.002	0	0	0	0	0.012
SM (OH) C22:1	sphingolipids	FIA	0.018	0.019	0.036	0.058	0.002	0.018
SM (OH) C22:2	sphingolipids	FIA	0.007	0.006	0.009	0.108	0	0.023
SM (OH) C24:1	sphingolipids	FIA	0.007	0.016	0.005	0.017	0.006	0.002
SM C16:0	sphingolipids	FIA	0.083	0.041	0.086	0.101	0.009	0.048
SM C16:1	sphingolipids	FIA	0.015	0.015	0.008	0.056	0	0.059
SM C18:0	sphingolipids	FIA	0.023	0.012	0.043	0.042	0.006	0.021
SM C18:1	sphingolipids	FIA	0.006	0.001	0.011	0.013	0.004	0.004
SM C20:2	sphingolipids	FIA	0.003	0.015	0.006	0.007	0	0.011
SM C22:3	sphingolipids	FIA	0.004	0.005	0.008	0	0	0
SM C24:0	sphingolipids	FIA	0.099	0.06	0.14	0.14	0.006	0.205
SM C24:1	sphingolipids	FIA	0.028	0.009	0.1	0.065	0	0.043
SM C26:0	sphingolipids	FIA	0.004	0.003	0.005	0.009	0	0.008
SM C26:1	sphingolipids	FIA	0.005	0.007	0.005	0.011	0	0.003
H1	sugars	FIA	45.8	30.4	22.7	151	28.8	1118

Supplementary Table S-16. Concentrations of standards used for calibration curves (μM).

Calibration Standard Concentrations (μM)							
Analyte	Calibrator level						
	Cal-1	Cal-2	Cal-3	Cal-4	Cal-5	Cal-6	Cal-7
Ala	20	40	200	400	800	1200	1600
Arg	5	10	50	100	200	300	400
Asn	5	10	50	100	200	300	400
Asp	5	10	50	100	200	300	400
Cit	5	10	50	100	200	300	400
Gln	20	40	200	400	800	1200	1600
Glu	10	20	100	200	400	600	800
Gly	25	50	250	500	1000	1500	2000
His	5	10	50	100	200	300	400
Ile	5	10	50	100	200	300	400
Leu	5	10	50	100	200	300	400
Lys	10	20	100	200	400	600	800
Met	5	10	50	100	200	300	400
Orn	5	10	50	100	200	300	400
Phe	5	10	50	100	200	300	400
Pro	10	20	100	200	400	600	800
Ser	5	10	50	100	200	300	400
Thr	5	10	50	100	200	300	400
Trp	5	10	50	100	200	300	400
Tyr	5	10	50	100	200	300	400
Val	10	20	100	200	400	600	800
Ac-Orn	0.5	1	5	10	20	30	40
ADMA	0.25	0.5	2.5	5	10	15	20
SDMA (HPLC)	1	2	10	20	40	60	80
SDMA (UHPLC)	0.1	0.2	1	2	3	6	8
alpha-AAA	1	2	10	20	40	60	80
Carnosine	0.5	1	5	10	20	30	40
Creatinine	10	20	100	200	400	600	800
DOPA	0.5	1	5	10	20	30	40
Dopamine	1	2	10	20	40	60	80
Histamine	1	2	10	20	40	60	80
Kynurenine	1	2	10	20	40	60	80
Met-SO	1	2	10	20	40	60	80
Nitro-Tyr	1	2	10	20	40	60	80
c4-OH-Pro	1	2	10	20	40	60	80
t4-OH-Pro	1	2	10	20	40	60	80
PEA	0.1	0.2	1	2	4	6	8
Putrescine	0.1	0.2	1	2	4	6	8
Serotonin	0.1	0.2	1	2	4	6	8
Spermidine	0.25	0.5	2.5	5	10	15	20
Spermine	0.25	0.5	2.5	5	10	15	20
Taurine	2.5	5	25	50	100	150	200
total DMA (HPLC only)	1.25	2.5	12.5	25	50	75	100
Sarcosine (UHPLC only)	1	2	10	20	40	60	80

Supplementary Table S-17. Intra-lab CVs calculated with the requirement of minimum $n=3$ and $n=2$ for the 20 test material. Out of a total 22680 possible intra-laboratory CV values (189 metabolites $\times$ 6 laboratories $\times$ 20 test material), 453 values could not be calculated because of the requirement of $n=3$, compared with $n=2$. Most of these values (383 values) were in laboratory D, mainly because of the exclusion of the two outlier samples TS05-D-17 and TS07-3-D-90.

Supplementary Table S-17

	n=2						n=3					
	LAB A	LAB B	LAB C	LAB D	LAB E	LAB F	LAB A	LAB B	LAB C	LAB D	LAB E	LAB F
	median CV	median CV	median CV	median CV	median CV	median CV	median CV	median CV	median CV	median CV	median CV	median CV
C0	4.0	5.0	4.1	6.9	5.1	7.1	4.0	5.0	4.1	6.9	5.1	7.1
C10	5.0	5.1	4.7	5.8	5.7	8.4	5.0	5.1	4.7	5.8	5.7	8.4
C10:1	7.1	6.3	5.9	8.3	10.9	11.8	7.1	6.3	5.9	8.1	10.9	11.8
C10:2	7.6	5.7	7.3	9.3	9.4	6.1	7.6	5.7	7.3	9.3	9.4	6.1
C12	5.9	5.8	6.6	7.5	6.5	7.2	5.9	5.8	6.6	8.1	6.5	7.2
C12-DC	6.6	5.3	3.7	6.2	5.3	5.1	6.6	5.3	3.7	6.2	5.3	5.1
C12:1	7.6	6.2	7.0	8.7	9.9	9.2	7.6	6.2	7.0	8.7	9.9	9.2
C14	6.1	7.4	4.2	7.8	7.5	5.8	6.1	7.4	4.2	8.2	7.5	5.8
C14:1	5.1	6.6	5.5	7.2	5.3	7.6	5.1	6.6	5.5	7.9	5.3	7.6
C14:1-OH	7.4	7.6	8.6	8.8	10.0	7.9	7.4	7.6	8.6	9.1	10.0	7.9
C14:2	7.5	7.9	6.2	10.4	8.0	7.3	7.5	7.9	6.2	10.4	8.0	7.3
C14:2-OH	7.3	8.9	12.4	11.6	12.7	10.4	7.3	8.9	12.4	13.7	12.7	10.4
C16	4.6	5.1	5.8	6.5	5.3	6.5	4.6	5.1	5.8	6.8	5.3	6.5
C16-OH	8.5	11.5	9.1	8.3	14.2	8.8	8.5	11.5	9.1	6.5	14.2	8.8
C16:1	6.4	7.5	5.3	7.4	4.5	8.1	6.4	7.5	5.3	6.9	4.5	8.1
C16:1-OH	8.6	7.9	10.9	8.2	10.8	7.6	8.6	7.9	10.9	8.3	10.8	7.6
C16:2	11.2	10.4	10.9	10.7	13.6	8.5	11.2	10.4	10.9	12.5	13.6	8.5
C16:2-OH	8.9	8.1	8.2	10.3	12.3	9.7	8.9	8.1	8.2	9.6	12.3	9.7
C18	5.9	8.4	7.4	7.7	7.1	9.6	5.9	8.4	7.4	8.4	7.1	9.6
C18:1	6.3	5.4	4.4	6.1	5.1	7.0	6.3	5.4	4.4	6.1	5.1	7.0
C18:1-OH	7.8	12.5	6.6	7.8	13.5	7.9	7.8	12.5	6.6	7.8	13.5	7.9
C18:2	7.5	8.0	5.6	9.8	7.1	8.8	7.5	8.0	5.6	9.8	7.1	8.8
C2	3.9	4.5	4.4	5.9	5.0	6.6	3.9	4.5	4.4	5.9	5.0	6.6
C3	4.6	5.6	5.2	5.5	6.4	7.8	4.6	5.6	5.2	5.5	6.4	7.8
C3:1	7.2	7.9	18.5	8.7	19.5	9.7	7.2	7.9	18.5	9.4	19.5	9.7
C3-DC (C4-OH)	6.0	7.1	6.7	6.8	9.0	12.2	6.0	7.1	6.7	6.8	9.0	12.2
C5-OH (C3-DC-M)	9.1	5.9	7.3	6.5	11.5	7.2	9.1	5.9	7.3	6.5	11.5	7.2
C3-OH	5.3	8.5	10.7	6.1	14.7	10.8	5.3	8.5	10.7	5.9	14.7	10.8
C4	5.9	6.8	5.9	7.3	7.3	7.0	5.9	6.8	5.9	8.0	7.3	7.0
C4:1	5.7	7.5	10.2	9.9	12.3	11.0	5.7	7.5	10.2	9.9	12.3	11.0
C5	5.1	5.9	5.9	7.2	3.5	8.8	5.1	5.9	5.9	7.9	3.5	8.8
C5:1	5.2	8.0	10.4	8.3	12.0	7.9	5.2	8.0	10.4	8.3	12.0	7.9
C5:1-DC	9.0	7.2	13.6	8.7	13.7	11.5	9.0	7.2	13.6	8.9	13.7	11.5
C5-DC (C6-OH)	10.1	7.1	10.0	10.9	10.0	8.2	10.1	7.1	10.0	11.9	10.0	8.2
C5-M-DC	8.4	9.5	10.4	6.0	11.5	7.2	8.4	9.5	10.4	6.0	11.5	7.2
C6:1	7.2	7.8	9.6	9.8	12.3	9.5	7.2	7.8	9.6	9.8	12.3	9.5
C6 (C4:1-DC)	6.2	6.2	6.2	7.0	6.4	7.6	6.2	6.2	6.2	7.0	6.4	7.6
C7-DC	6.4	8.2	8.5	9.9	9.1	10.5	6.4	8.2	8.5	9.9	9.1	10.5
C8	5.4	5.4	3.5	5.7	5.6	8.1	5.4	5.4	3.5	6.0	5.6	8.1
C9	6.1	6.2	6.3	7.4	8.8	6.0	6.1	6.2	6.3	7.4	8.8	6.0
lysoPC a C14:0	3.5	2.9	2.2	5.3	3.4	5.6	3.5	2.9	2.2	5.3	3.4	5.6
lysoPC a C16:0	5.6	5.9	4.0	8.0	4.6	7.7	5.6	5.9	4.0	8.1	4.6	7.7
lysoPC a C16:1	4.9	4.6	4.8	6.9	5.8	6.4	4.9	4.6	4.8	6.9	5.8	6.4
lysoPC a C17:0	5.2	5.2	6.4	8.6	5.7	8.2	5.2	5.2	6.4	8.6	5.7	8.2
lysoPC a C18:0	6.2	5.0	4.8	7.1	4.1	8.9	6.2	5.0	4.8	7.9	4.1	8.9
lysoPC a C18:1	5.6	5.6	4.1	8.3	4.0	8.0	5.6	5.6	4.1	8.4	4.0	8.0
lysoPC a C18:2	4.9	4.9	4.6	6.5	3.4	7.0	4.9	4.9	4.6	6.5	3.4	7.0
lysoPC a C20:3	5.8	4.8	5.3	8.2	4.4	8.1	5.8	4.8	5.3	8.4	4.4	8.1
lysoPC a C20:4	6.6	5.0	5.0	7.7	4.4	8.0	6.6	5.0	5.0	7.7	4.4	8.0
lysoPC a C24:0	6.9	16.4	12.0	18.3	14.2	13.4	6.9	16.4	12.0	18.4	14.2	13.4
lysoPC a C26:0	12.7	16.2	20.6	21.3	20.8	15.9	12.7	16.2	20.6	21.3	20.8	15.9
lysoPC a C26:1	12.3	18.9	20.0	16.8	20.6	15.1	12.3	18.9	20.0	18.6	20.6	15.1
lysoPC a C28:0	11.0	8.7	11.1	19.6	16.7	11.7	11.0	8.7	11.1	19.6	16.7	11.7
lysoPC a C28:1	6.4	7.4	7.9	13.1	9.3	11.3	6.4	7.4	7.9	13.1	9.3	11.3
PC aa C24:0	6.9	15.7	13.9	19.8	11.8	11.5	6.9	15.7	13.9	19.8	11.8	11.5
PC aa C26:0	4.9	11.2	5.7	16.6	5.4	12.6	4.9	11.2	5.7	16.6	5.4	12.6
PC aa C28:1	5.3	6.6	4.7	6.5	4.6	6.8	5.3	6.6	4.7	6.5	4.6	6.8
PC aa C30:0	4.5	5.6	3.4	6.0	5.4	6.9	4.5	5.6	3.4	6.0	5.4	6.9
PC aa C30:2	59.1	38.7	5.9	71.7	51.7	NQ	54.3	39.9	5.9	67.1	52.7	NA
PC aa C32:0	5.6	5.2	3.8	5.8	4.7	7.0	5.6	5.2	3.8	5.8	4.7	7.0
PC aa C32:1	5.3	4.6	3.4	9.6	4.5	6.7	5.3	4.6	3.4	9.6	4.5	6.7
PC aa C32:2	4.5	6.0	4.3	7.2	5.1	NQ	4.5	6.0	4.3	7.0	5.1	NA
PC aa C32:3	6.1	6.3	4.7	6.7	3.7	7.0	6.1	6.3	4.7	6.7	3.7	7.0
PC aa C34:1	4.7	6.1	3.8	5.1	4.5	7.2	4.7	6.1	3.8	5.1	4.5	7.2
PC aa C34:2	4.7	4.5	3.7	5.3	4.8	7.5	4.7	4.5	3.7	5.3	4.8	7.5
PC aa C34:3	5.2	4.6	4.4	5.3	5.4	7.0	5.2	4.6	4.4	5.3	5.4	7.0
PC aa C34:4	5.2	5.0	4.3	7.3	4.4	6.9	5.2	5.0	4.3	7.4	4.4	6.9
PC aa C36:0	5.4	7.4	4.4	14.6	7.4	6.7	5.4	7.4	4.4	13.0	7.4	6.7
PC aa C36:1	5.6	5.0	3.6	6.3	5.4	7.2	5.6	5.0	3.6	6.3	5.4	7.2
PC aa C36:2	4.1	4.4	4.2	5.8	4.3	7.5	4.1	4.4	4.2	5.8	4.3	7.5

Supplementary Table S-17

	n=2						n=3					
	LAB A	LAB B	LAB C	LAB D	LAB E	LAB F	LAB A	LAB B	LAB C	LAB D	LAB E	LAB F
	median CV	median CV	median CV	median CV	median CV	median CV	median CV	median CV	median CV	median CV	median CV	median CV
PC aa C36:3	5.0	5.5	4.1	5.3	4.4	6.6	5.0	5.5	4.1	5.3	4.4	6.6
PC aa C36:4	4.7	4.9	3.9	5.0	4.7	6.9	4.7	4.9	3.9	5.0	4.7	6.9
PC aa C36:5	5.0	5.4	3.6	6.0	4.3	7.4	5.0	5.4	3.6	6.0	4.3	7.4
PC aa C36:6	4.6	5.4	4.3	7.2	5.4	7.6	4.6	5.4	4.3	9.4	5.4	7.6
PC aa C38:0	5.2	5.1	4.5	6.0	3.5	7.6	5.2	5.1	4.5	6.0	3.5	7.6
PC aa C38:1	19.8	15.4	13.1	25.1	17.9	NQ	19.8	15.4	13.1	26.4	17.9	NA
PC aa C38:3	3.6	5.0	4.1	6.5	4.5	8.0	3.6	5.0	4.1	5.9	4.5	8.0
PC aa C38:4	3.6	4.5	4.1	5.0	4.6	7.4	3.6	4.5	4.1	5.9	4.6	7.4
PC aa C38:5	4.4	4.8	3.7	6.9	3.9	6.8	4.4	4.8	3.7	6.9	3.9	6.8
PC aa C38:6	4.4	4.8	4.0	7.6	4.3	8.1	4.4	4.8	4.0	8.0	4.3	8.1
PC aa C40:1	6.1	5.0	5.0	7.9	6.1	6.3	6.1	5.0	5.0	7.1	6.1	6.3
PC aa C40:2	7.0	7.9	7.7	9.1	6.3	7.0	7.0	7.9	7.7	9.1	6.3	7.0
PC aa C40:3	6.6	6.7	6.4	8.9	6.2	6.5	6.6	6.7	6.4	9.4	6.2	6.5
PC aa C40:4	4.5	5.8	4.4	8.2	5.8	6.8	4.5	5.8	4.4	8.2	5.8	6.8
PC aa C40:5	4.9	5.9	4.2	6.2	4.5	7.0	4.9	5.9	4.2	6.2	4.5	7.0
PC aa C40:6	4.8	5.1	3.7	5.2	4.3	7.5	4.8	5.1	3.7	5.2	4.3	7.5
PC aa C42:0	6.1	6.4	4.8	7.6	5.1	7.9	6.1	6.4	4.8	7.4	5.1	7.9
PC aa C42:1	4.7	6.5	8.2	9.6	4.3	6.9	4.7	6.5	8.2	8.7	4.3	6.9
PC aa C42:2	6.0	5.8	6.1	8.4	7.2	6.7	6.0	5.8	6.1	9.2	7.2	6.7
PC aa C42:4	9.4	10.7	8.9	10.6	6.9	6.8	9.4	10.7	8.9	10.9	6.9	6.8
PC aa C42:5	6.7	8.1	6.8	8.4	6.6	7.7	6.7	8.1	6.8	8.4	6.6	7.7
PC aa C42:6	5.3	6.0	5.5	8.8	5.1	5.4	5.3	6.0	5.5	8.4	5.1	5.4
PC ae C30:0	4.8	5.6	7.2	7.6	4.9	7.1	4.8	5.6	7.2	7.7	4.9	7.1
PC ae C30:1	41.8	27.3	16.1	25.7	23.9	43.0	44.1	27.3	16.1	26.7	24.9	43.0
PC ae C30:2	6.3	8.9	6.8	9.4	8.3	8.8	6.3	8.9	6.8	9.7	8.3	8.8
PC ae C32:1	6.5	5.0	4.4	7.0	4.8	7.6	6.5	5.0	4.4	7.9	4.8	7.6
PC ae C32:2	5.6	5.2	5.4	8.6	5.0	6.9	5.6	5.2	5.4	8.6	5.0	6.9
PC ae C34:0	6.3	6.3	3.8	6.6	4.7	6.3	6.3	6.3	3.8	6.7	4.7	6.3
PC ae C34:1	5.5	5.2	3.6	6.6	4.9	7.2	5.5	5.2	3.6	6.6	4.9	7.2
PC ae C34:2	5.3	5.1	3.9	6.7	4.7	7.4	5.3	5.1	3.9	6.7	4.7	7.4
PC ae C34:3	4.6	4.3	4.0	5.9	5.1	8.1	4.6	4.3	4.0	5.9	5.1	8.1
PC ae C36:0	6.6	6.3	5.4	10.8	8.8	4.5	6.6	6.3	5.4	10.5	8.8	4.5
PC ae C36:1	4.5	5.3	3.4	6.8	4.3	7.4	4.5	5.3	3.4	6.8	4.3	7.4
PC ae C36:2	4.7	5.2	3.3	6.0	4.3	7.8	4.7	5.2	3.3	6.0	4.3	7.8
PC ae C36:3	5.2	4.7	4.0	6.7	4.1	7.7	5.2	4.7	4.0	6.7	4.1	7.7
PC ae C36:4	4.9	4.4	3.7	6.7	4.4	8.4	4.9	4.4	3.7	6.7	4.4	8.4
PC ae C36:5	5.8	5.1	4.0	7.1	5.0	8.3	5.8	5.1	4.0	7.1	5.0	8.3
PC ae C38:0	4.8	4.3	3.5	7.4	4.4	7.7	4.8	4.3	3.5	7.4	4.4	7.7
PC ae C38:1	37.1	19.8	20.2	26.4	13.1	NA	30.7	19.8	20.2	25.6	13.1	NA
PC ae C38:2	5.8	6.2	5.4	13.4	6.4	9.9	5.8	6.2	5.4	14.4	6.4	9.9
PC ae C38:3	4.7	4.9	4.4	5.2	3.5	7.4	4.7	4.9	4.4	5.2	3.5	7.4
PC ae C38:4	5.0	4.4	4.5	5.4	5.1	7.5	5.0	4.4	4.5	5.4	5.1	7.5
PC ae C38:5	4.7	5.0	4.3	6.2	4.8	7.9	4.7	5.0	4.3	6.2	4.8	7.9
PC ae C38:6	5.6	5.5	4.3	6.4	3.8	8.0	5.6	5.5	4.3	6.4	3.8	8.0
PC ae C40:1	6.4	4.9	5.1	12.1	4.6	6.4	6.4	4.9	5.1	12.1	4.6	6.4
PC ae C40:2	5.1	4.6	4.4	7.3	4.9	7.1	5.1	4.6	4.4	7.3	4.9	7.1
PC ae C40:3	4.1	4.7	5.7	8.6	5.6	6.6	4.1	4.7	5.7	8.6	5.6	6.6
PC ae C40:4	4.7	5.3	4.3	6.4	6.1	7.6	4.7	5.3	4.3	6.4	6.1	7.6
PC ae C40:5	4.2	4.8	4.7	7.5	4.3	8.0	4.2	4.8	4.7	7.5	4.3	8.0
PC ae C40:6	4.5	5.0	3.9	7.1	4.5	7.0	4.5	5.0	3.9	7.1	4.5	7.0
PC ae C42:0	3.2	4.5	3.3	4.0	3.5	4.1	3.2	4.5	3.3	3.7	3.5	4.1
PC ae C42:1	4.9	8.5	6.5	13.4	9.3	5.7	4.9	8.5	6.5	13.4	9.3	5.7
PC ae C42:2	4.5	6.7	6.5	10.5	4.7	7.3	4.5	6.7	6.5	10.1	4.7	7.3
PC ae C42:3	6.3	7.2	4.8	10.1	6.2	5.7	6.3	7.2	4.8	10.1	6.2	5.7
PC ae C42:4	5.8	6.6	5.1	9.0	5.2	7.7	5.8	6.6	5.1	9.5	5.2	7.7
PC ae C42:5	3.8	4.2	5.1	6.3	5.1	6.2	3.8	4.2	5.1	5.3	5.1	6.2
PC ae C44:3	8.2	6.9	11.6	14.0	12.0	5.9	8.2	6.9	11.6	13.5	12.0	5.9
PC ae C44:4	6.2	7.4	6.0	10.7	6.0	5.6	6.2	7.4	6.0	11.4	6.0	5.6
PC ae C44:5	5.1	6.1	6.1	6.7	4.9	6.5	5.1	6.1	6.1	6.7	4.9	6.5
PC ae C44:6	3.6	4.4	3.3	6.6	4.7	7.9	3.6	4.4	3.3	5.5	4.7	7.9
SM (OH) C14:1	4.5	4.7	4.7	7.6	4.9	6.7	4.5	4.7	4.7	8.1	4.9	6.7
SM (OH) C16:1	4.9	5.3	5.0	7.1	4.8	7.9	4.9	5.3	5.0	8.7	4.8	7.9
SM (OH) C22:1	5.9	4.5	4.6	8.0	5.4	6.9	5.9	4.5	4.6	8.2	5.4	6.9
SM (OH) C22:2	6.6	4.3	5.1	8.7	4.0	7.3	6.6	4.3	5.1	7.9	4.0	7.3
SM (OH) C24:1	7.9	7.7	6.5	7.0	7.1	7.4	7.9	7.7	6.5	7.0	7.1	7.4
SM C16:0	4.6	4.6	4.2	6.6	4.5	6.8	4.6	4.6	4.2	7.6	4.5	6.8
SM C16:1	5.4	5.3	4.7	7.3	4.2	6.4	5.4	5.3	4.7	7.3	4.2	6.4
SM C18:0	4.5	4.7	3.8	7.0	4.8	7.4	4.5	4.7	3.8	7.1	4.8	7.4
SM C18:1	5.1	5.2	3.8	5.7	5.1	5.7	5.1	5.2	3.8	6.9	5.1	5.7
SM C20:2	8.8	11.3	5.1	11.9	13.1	7.5	8.8	11.3	5.1	11.9	13.1	7.5

Supplementary Table S-17

	n=2						n=3					
	LAB A	LAB B	LAB C	LAB D	LAB E	LAB F	LAB A	LAB B	LAB C	LAB D	LAB E	LAB F
	median CV	median CV	median CV	median CV	median CV	median CV	median CV	median CV	median CV	median CV	median CV	median CV
SM C22:3	59.0	60.4	5.0	9.4	131.9	NQ	NA	75.3	5.0	NA	NA	NA
SM C24:0	5.6	4.7	4.4	6.6	5.0	7.2	5.6	4.7	4.4	6.4	5.0	7.2
SM C24:1	5.9	4.9	4.6	7.4	4.8	8.0	5.9	4.9	4.6	7.9	4.8	8.0
SM C26:0	10.2	20.1	16.8	19.7	10.8	11.0	10.2	20.1	16.8	19.7	10.8	11.0
SM C26:1	8.0	11.2	7.2	15.0	11.8	8.4	8.0	11.2	7.2	15.0	11.8	8.4
H1	4.5	5.2	4.4	6.5	4.3	11.5	4.5	5.2	4.4	6.5	4.3	11.6
Ala	1.8	2.1	3.3	4.1	4.2	4.5	1.8	2.1	3.3	4.1	4.2	4.5
Arg	5.0	5.7	5.1	6.2	8.5	8.3	5.0	5.7	5.1	6.2	8.5	8.3
Asn	3.4	2.4	4.6	4.4	6.1	6.4	3.4	2.4	4.6	4.7	6.1	6.4
Asp	11.2	10.8	21.6	24.2	14.3	17.4	11.6	10.8	21.6	24.4	14.3	17.4
Cit	4.0	3.2	5.8	5.8	11.6	6.5	4.0	3.2	5.8	6.4	11.6	6.5
Gln	5.0	4.7	5.6	7.9	8.5	6.6	5.0	4.7	5.6	7.9	8.5	6.6
Glu	4.1	3.1	6.6	6.6	8.4	6.8	4.1	3.1	6.6	7.1	8.4	6.8
Gly	4.4	4.5	6.5	6.1	5.5	7.1	4.4	4.5	6.5	6.1	5.5	7.1
His	4.5	5.8	5.6	5.9	4.8	6.8	4.5	5.8	5.6	5.9	4.8	6.8
Ile	3.7	5.2	5.2	6.1	4.0	6.9	3.7	5.2	5.2	6.1	4.0	6.9
Leu	4.0	4.1	5.8	7.0	4.8	7.9	4.0	4.1	5.8	7.0	4.8	7.9
Lys	4.8	5.3	6.8	6.4	6.3	6.6	4.8	5.3	6.8	6.4	6.3	6.6
Met	4.8	4.8	7.0	6.2	5.8	7.0	4.8	4.8	7.0	6.3	5.8	7.0
Orn	4.4	5.6	6.1	7.6	8.2	7.3	4.4	5.6	6.1	7.6	8.2	7.3
Phe	3.7	5.2	5.0	6.0	4.1	9.1	3.7	5.2	5.0	6.0	4.1	9.1
Pro	4.6	5.0	5.8	5.3	4.5	7.9	4.6	5.0	5.8	6.6	4.5	7.9
Ser	4.7	5.1	7.5	8.3	8.8	7.3	4.7	5.1	7.5	8.3	8.8	7.3
Thr	2.2	2.4	4.1	3.7	8.4	4.6	2.2	2.4	4.1	3.9	8.4	4.6
Trp	4.7	4.5	6.0	6.4	5.6	8.1	4.7	4.5	6.0	6.4	5.6	8.1
Tyr	3.9	5.0	7.0	7.5	4.7	8.3	3.9	5.0	7.0	7.5	4.7	8.3
Val	4.1	4.2	5.6	6.1	4.8	7.2	4.1	4.2	5.6	6.1	4.8	7.2
Ac-Orn	NA	4.7	7.6	10.1	6.6	2.8	NA	4.7	7.6	11.2	6.6	2.8
ADMA	14.2	5.4	11.2	12.7	17.9	19.7	14.2	5.4	11.2	12.7	17.9	19.7
alpha-AAA	11.6	10.0	15.9	42.7	12.2	13.6	11.6	10.0	15.9	42.7	12.2	13.6
c4-OH-Pro	NA	NA	NA	6.0	9.1	NA	NA	NA	NA	7.2	9.1	NA
Carnosine	3.5	8.9	NA	0.5	5.1	NA	3.5	8.9	NA	0.5	NA	NA
Creatinine	1.8	2.6	2.6	3.7	3.5	49.7	1.8	2.6	2.6	3.9	3.5	49.7
DOPA	0.7	4.0	NA	3.4	50.1	2.9	NA	4.0	NA	3.1	112.1	2.9
Dopamine	NA	NA	NA	10.4	NA	NA	NA	NA	NA	11.0	NA	NA
Histamine	0.1	0.1	0.3	NA	0.3	NA	0.1	0.1	0.3	NA	0.3	NA
Kynurenine	3.8	5.5	6.7	6.9	6.7	7.1	3.8	5.5	6.7	6.9	6.7	7.1
Met-SO	12.1	7.5	8.1	9.0	18.2	48.9	12.1	7.5	8.1	9.0	18.2	26.9
Nitro-Tyr	1.6	NA	NA	16.4	NA	NA	NA	NA	NA	14.4	NA	NA
PEA	NA	NA	NA	49.8	NA	NA	NA	NA	NA	49.8	NA	NA
Putrescine	14.7	10.3	7.1	NA	15.3	11.4	14.7	10.3	7.1	NA	15.9	30.1
Sarcosine	NQ	NQ	NQ	8.9	NQ	6.1	NA	NA	NA	8.9	NA	6.1
SDMA	64.5	7.7	19.6	7.2	26.9	9.2	81.2	7.7	19.6	7.4	26.9	9.2
Serotonin	4.1	6.4	5.1	8.3	7.1	6.9	4.1	6.4	5.1	8.8	7.1	6.9
Spermidine	1.6	1.6	3.1	7.0	2.8	4.4	1.6	1.6	3.1	7.4	2.8	4.4
Spermine	1.3	1.1	1.5	21.4	2.9	5.7	1.3	1.1	1.5	21.4	2.9	6.5
t4-OH-Pro	3.7	6.5	7.7	6.2	7.9	8.8	3.7	6.5	7.7	6.4	7.9	8.8
Taurine	2.8	1.6	3.9	3.9	6.2	5.6	2.8	1.6	3.9	3.9	6.2	5.6
total DMA	4.7	3.2	8.3	NQ	17.3	NQ	4.7	3.2	8.3	NA	17.3	NA

Supplementary Protocol S-1. Basic common guidance and checklist for instrument cleaning and benchmarking prior to analysis

Ring Trial Absolute/DQ® p180 Kit

– Instructions and Checklist for Preparing your LC-MS System –

Dear Participants,

Thank you for taking part in the p180 Kit Ring Trial. Before you start with the Kit, it is required to make preparations at your instrumentation. This checklist gives instructions for cleaning and checking your system performance. The following instruments are recommended:

Mass Spectrometer: AB SCIEX 4000 and 5500 triple quadrupole series
Waters Xevo TQ-MS and TQ-S
Thermo TSQ Vantage

LC System: Binary pump system (HPLC or UHPLC) with degasser unit and column oven

Autosampler: A 96-wellplate autosampler with temperature control (10°C).
Injection Volumes: FIA 20 µL, HPLC 10 µL (5 µL if the UHPLC method is applied)

The latest service of your instrument may not be older than 6 months. All solvents must be HPLC grade and should be sonicated for 10 min. Please mind to wash all solvent bottles at least with water and methanol before use.

Please complete the following steps directly before you start with the Kit:

- ✚ Clean the following device at your MS system, depending on the manufacturer:
 - Curtain Plate (AB SCIEX) / Ion Sweep Cone and Ion Transfer Capillary (Thermo): use metal polish and a paper tissue. Then sonicate the device in 50% isopropanol (in MilliQ water) for 10 min, followed by another 10 min in pure methanol. Dry the device in the air or by compressed air / nitrogen.
 - Cone (Waters): use a cotton swab dipped in 100% formic acid. Then sonicate the device two times for 5 min in MilliQ water, followed by another 10 min in pure methanol. Dry the device in the air or by compressed air / nitrogen.
- ✚ Install 90% methanol (in MilliQ water) at your LC system, put both solvent lines (A and B) into it and purge. Then turn on the ion spray and flush the whole system for at least 15 min at a flow rate of 1.0 mL/min.
- ✚ Install the wash solvents according to the p180 user manual (page 14). Purge and flush the entire injection system. Pipette 90% methanol (in MilliQ water) into a sample vial and inject at least ten times (maximum injection volume). Select 50% A and 50% B in the gradient method (both lines still in 90% methanol). Use an isocratic flow rate of 1.0 mL/min.
- ✚ You find two Testmix vials in the Kit box, one for the LC part ("KIT2/MD01 Testmix") and one for the FIA part ("KIT1/KIT3 Testmix"), as well as instruction sheets. Before you start with the LC part, run the corresponding Testmix and check the column condition and instrument sensitivity.

Before you run the FIA plate, it is important to perform the system test strictly according to the instruction sheet using the MetIDQ project template. A proper Kit performance can only be guaranteed when the autosampler is clean and the blank levels are low. The FIA part is more affected by autosampler contaminations, because more than 100 lipids are analyzed and autosamplers are often contaminated with lipids. If necessary, clean the autosampler parts such as syringe, sample loop and switching valve.

Additional remarks:

- ✚ On the USB stick you find the MetIDQ project for the ring trial, which already contains the LC and FIA worklist with the official plate layout (file names: *p180 ring trial_Sciex.metidq* and *p180 ring trial_Waters.metidq*). Import the project (Waters or Sciex, depending on your MS platform) into MetIDQ using the "Import Project" button. Change the OP for the LC and FIA plate if necessary (according to the OPs that you are currently using for the Kit). Continue and prepare the Kit according to the Absolute/DQ p180 user manual.
- ✚ Fresh phenylisothiocyanate and pyridine (unopened bottles) must be used for preparing the derivatization solution.
- ✚ Use an Eppendorf Multipette for pipetting the additional internal standard (ISTD vial) and the derivatization solution.
- ✚ Pipette the Kit plate according to the official plate layout. In summary:
 - Blank
 - Zero (PBS), 3 replicates
 - Calibration Standards, 7 levels
 - Biocrates QC 1, barcode 438006, 5 replicates (3x beginning, 2x end of plate)
 - Biocrates QC 2, barcode 438010, 5 replicates (3x beginning, 2x end of plate)
 - Biocrates QC 3, barcode 438023, 3 replicates (3x beginning of plate)
 - Biocrates Sample 1 to 6, each in replicates of 3
 - Test Sample 07-1 to 10-4, each in replicates of 3
 - QC Pool (pool of all Test Samples 07-1 to 10-4), each in replicates of 6
- ✚ Vortex the samples after thawing and centrifuge at 4°C for 5 minutes at 2750 x g. Keep the samples on ice afterwards. They may not be kept at room temperature for longer time (stability issues of several metabolites).
- ✚ Prepare the QC Pool: Transfer 10 µL of each Test Sample (07-1 to 10-4) to the empty vial with the label "QC Pool". Vortex vigorously.

Your Support Team

Supplementary Protocol S-2. Metabolomics measurements - Laboratory instrumentation.

Metabolomics measurements

Basic common guidance (Protocol S-1) was agreed on the cleaning and benchmarking of instruments prior to analysis, and also the run order and the position of samples in 96 well plates, with vertical pipetting and run order mode (Figure S-1). Plasma and serum metabolite concentrations were determined using the targeted metabolomics kit Absolute/IDQ™ p180 kit (BIOCRATES Life Sciences AG, Innsbruck, Austria). The samples were prepared, by all participating laboratories, according to the manufacture's protocol²². In short: serum or plasma were vortexed and centrifuged. A 10 µL solution of internal standards and 10 µL of serum or plasma samples were pipetted onto an inserted filter in a 96 well plate. The filters were dried in nitrogen stream, and 50 µL of derivatisation solution (5% phenylisothiocyanate reagent, PITC) were added and filters were dried again. After extraction of metabolites and internal standards with 5 mM ammonium acetate in methanol, the solution was centrifuged through the filter membrane. Two separate dilutions of the final extract were prepared for LC-MS/MS analysis (in MeOH/H₂O 1/1) and for FIA-MS/MS analysis (in FIA solvent).

Laboratory instrumentation – MS analysis

Each laboratory followed the manufacturer's protocol but used different UHPLC/HPLC or MS/MS platforms. Laboratories A, B, C and E used HPLC with SCIEX mass spectrometers. Lab F used Waters UHPLC with a Waters mass spectrometer. Lab D used a combination of Waters UHPLC with a SCIEX mass spectrometer.

Laboratory code	Analytical platform	Sample extract dilutions
Lab A	Agilent LC 1290 (HPLC) – SCIEX QTRAP 5500	LC (1:10), FIA (1:50)
Lab B	Agilent LC 1100 (HPLC) – SCIEX QTRAP 6500	LC (1:4), FIA (1:9)
Lab C	Agilent LC 1200 (HPLC) – SCIEX API 4000 Robot Hamilton Microlab STAR™.	LC (1:2), FIA (1:6)
Lab D	Waters Acquity UHPLC H-Class (UHPLC) – SCIEX QTRAP 5500.	LC (1:10), FIA (1:50)
Lab E	For LC acquisition, Agilent 1100 (HPLC) – SCIEX QTRAP 4000. For FIA acquisition, Agilent 1200 - SCIEX QTRAP 5500.	LC (1:2), FIA (1:50)
Lab F	Waters Acquity UHPLC I-Class (UHPLC) - Waters TQS	LC (1:10), FIA (1:20)

Two examples of typical MS analytical procedures are described in Protocol S-2, a combination of HPLC coupled with SCIEX mass spectrometer (Lab B) and a combination of Waters UHPLC coupled with Waters mass spectrometer (Lab F).

HPLC – SCIEX mass spectrometer

Mass spectrometric analyses were carried out on a SCIEX QTRAP 6500 equipped with an Agilent 1100 series HPLC controlled by the Analyst 1.6.2 software. For LC-MS/MS, 10 µL of sample preparation were injected on a reverse-phase column Agilent Zorbax Eclipse XDB C18, 3.0 x 100 mm, 3.5 µm with pre-column, SecurityGuard, Phenomenex, C18, 4 x 3 mm using an Agilent 1100 system, at a flow rate of 0.5 mL/min. The binary gradient elution system comprised of (A): water + 0.2% formic acid and (B) acetonitrile + 0.2% FA. The elution gradient profile was as follows (min / % of B): 0/0, 0.5/0, 5.5/95, 6.5/95, 7/0, 9.5/0. The column was kept at 50 °C and the auto-sampler at 10 °C. The column eluent was introduced directly into the mass spectrometer by electrospray ionization

and mass spectrometric analyses were carried out on a SCIEX QTRAP 6500 in positive ion mode. For FIA-MS/MS, 20 µL of sample preparation were injected directly into the mass spectrometer by electrospray ionization and mass spectrometric analyses were carried out on a SCIEX QTRAP 6500 in the positive and negative ion modes. The elution profile was as follows (min / flow-rate in µl/min): 0/30, 1.6/30, 2.4/200, 2.8/200, 3/30. The auto-sampler was kept at 10 °C.

UHPLC – Waters mass spectrometer

Mass spectrometric analyses were carried out on a Waters Acquity UPLC I-Class XEVO TQ-S controlled by the MassLynx 4.1 software. For LC-MS/MS, 5 µL of sample preparation were injected on a reverse-phase column Waters Acquity UPLC BEH C18 1.7 µm 2.1 x 50 mm with pre-column Waters Acquity UPLC BEH C18 1.7 µm VANGUARD at a flow rate of 0.9 mL/min. The binary gradient elution system comprised of (A): water + 0.2% formic acid and (B) acetonitrile + 0.2% FA. The elution gradient profile was as follows (min / % of B): 0/0, 0.25/0, 3.75/60, 4.05/0, 4.15/0. The column was kept at 50 °C and the auto-sampler at 10 °C. The column eluent was introduced directly into the mass spectrometer by electrospray ionization and mass spectrometric analyses were carried out on a XEVO TQ-S in the positive ion mode. For FIA-MS/MS, 20 µL of sample preparation were injected directly into the mass spectrometer by electrospray ionization and mass spectrometric analyses were carried out on a Waters XEVO TQ-S in the positive ion mode. The elution profile was as follows (min / flow-rate in µl/min): 0/30, 1.6/30, 2.4/200, 2.8/200, 3/30. The auto-sampler was kept at 10 °C.

Lab C: Used 20 µL derivatisation solution and for initial and final FIA flow-rate 35 µL/min.