LipidQC: Method Validation Tool for Visual Comparison to SRM 1950 Using NIST Interlaboratory Comparison Exercise Lipid Consensus Mean Estimate Values

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ABSTRACT: As advances in analytical separation techniques, mass spectrometry instrumentation, and data processing platforms continue to spur growth in the lipidomics field, more structurally unique lipid species are detected and annotated. The lipidomics community is in need of benchmark reference values to assess the validity of various lipidomics workflows in providing accurate quantitative measurements across the diverse lipidome. LipidQC addresses the harmonization challenge in lipid quantitation by providing a semi-automated process, independent of analytical platform, for visual comparison of experimental results of NIST SRM 1950 Metabolites in Frozen Human Plasma against benchmark consensus mean concentrations derived from the NIST Lipidomics Interlaboratory Comparison Exercise.

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FIGURES

Figure S-1: Example summary table for experimental results compared against SRM 1950 - "Metabolites in Frozen Human Plasma". The mean and standard deviation (measurement) are calculated from the user-generated experimental data and compared to the consensus mean value (nmol/mL). For the consensus value, the consensus mean is highlighted in blue and the corresponding uncertainty is highlighted in green. **[A]** Screen-capture of summary table for the data obtained from Bligh–Dyer lipid extracts analyzed by UHPLC-HRMS (Figure S1-A). **[B]** Screen-capture of summary table for data obtained from modified Bligh–Dyer lipid extracts analyzed by DI-MS/MS (Figure S1-B). Isobaric species within the same lipid class were summed and reported in the NIST Lipidomics Interlaboratory Study as a single consensus mean estimate value. Therefore, the summed isobaric lipid species present in the interlaboratory study (PC P-38:6/36:0) is shown in the table and the note indicates that the user only provided data for PC 36:0 in the input table from Figure S1-B.

[A]

Lipid Species	Measurement*	Consensus Value**	Units	No. of labs	Notes
TAG 50:0	4.629 ± 0.21	3.8 ± 0.83	nmol/mL	11	
TAG 50:4	6.044 ± 0.381	8.7 ± 2.9	nmol/mL	15	
TAG 52:3	114.9 ± 0.984	100 ± 29	nmol/mL	16	
LPC 20:4	6.483 ± 1.287	6.0 ± 0.60	nmol/mL	20	
LPC 22:1	0.012 ± 0.001	0.013 ± 0.0046	nmol/mL	5	
LPE 22:6	0.909 ± 0.02	0.52 ± 0.18	nmol/mL	12	
PC 34:0	2.646 ± 0.402	2.1 ± 0.37	nmol/mL	12	
PC 36:2	184 ± 19.51	140 ± 25	nmol/mL	18	
PC 38:3	28.36 ± 2.395	26 ± 5.2	nmol/mL	14	
PE 34:2	1.69 ± 0.076	2.2 ± 0.26	nmol/mL	16	
PE 38:4	5.89 ± 0.503	8.1 ± 1.2	nmol/mL	16	
SM d34:0	7.721 ± 1.305	5.8 ± 1.3	nmol/mL	14	
SM d34:2	15.41 ± 2.596	16 ± 2.2	nmol/mL	17	
LPC 24:1	0.013 ± 0.002	0.022 ± 0.0071	nmol/mL	3	

[B]

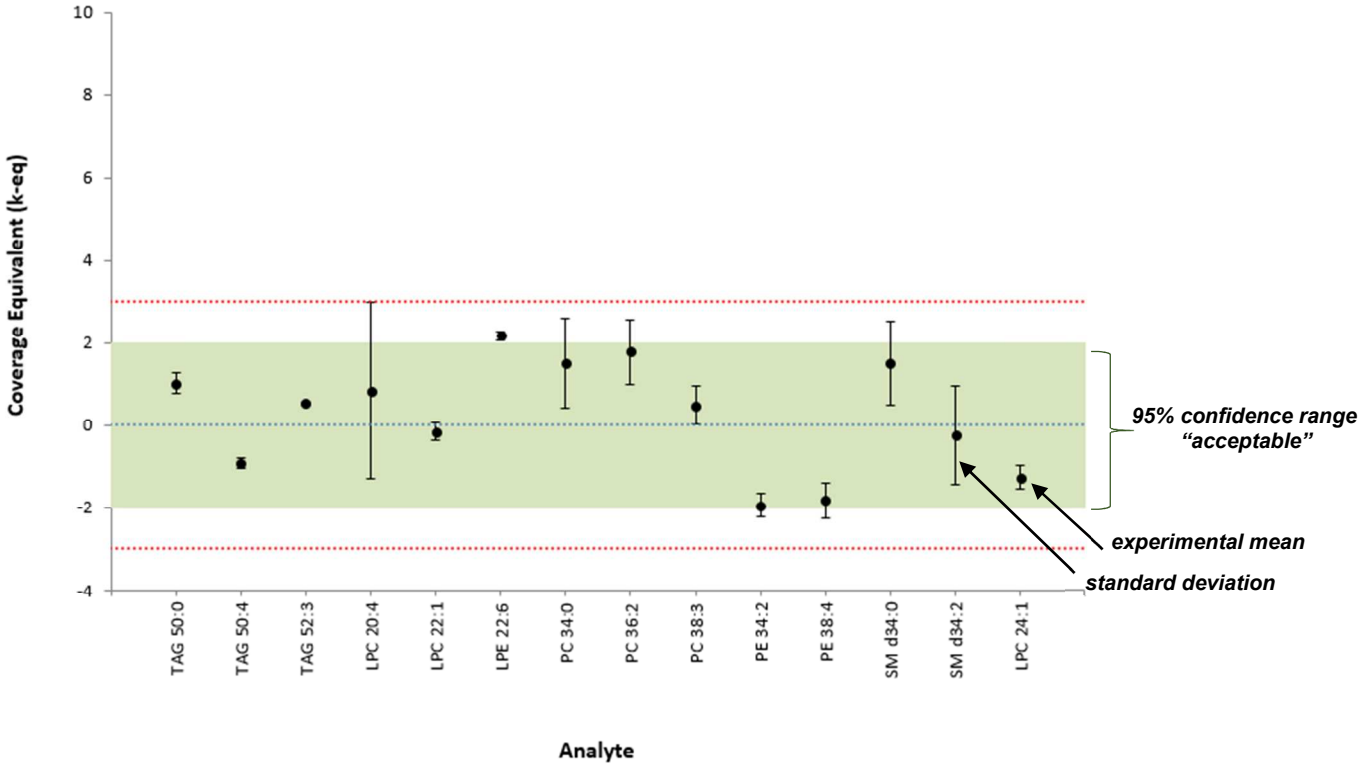
Lipid Species	Measurement*	Consensus Value**	Units	No. of labs	Notes
FFA 16:0	180.2 ± 10.08	43 ± 13	nmol/mL	5	
FFA 20:4	4.594 ± 0.75	4.7 ± 1.5	nmol/mL	7	
FFA 20:5	3.238 ± 0.059	0.42 ± 0.056	nmol/mL	7	
DAG 36:2	5.234 ± 0.267	6.2 ± 2.2	nmol/mL	16	
DAG 36:3	6.815 ± 1.283	8.4 ± 3.3	nmol/mL	15	
DAG 36:4	0.276 ± 0.046	2.8 ± 1.0	nmol/mL	12	
TAG 50:0	29.03 ± 0.745	3.8 ± 0.83	nmol/mL	11	
TAG 56:7	6.207 ± 0.163	13 ± 2.7	nmol/mL	8	
LPE 16:0	0.753 ± 0.027	0.91 ± 0.27	nmol/mL	14	
PC P-38:6/36:0	2.144 ± 0.057	1.2 ± 0.39	nmol/mL	10	Includes only PC 36:0 results.
CER d34:1	0.441 ± 0.075	0.28 ± 0.044	nmol/mL	17	
CER d36:1	0.139 ± 0.015	0.12 ± 0.021	nmol/mL	14	
SM d38:1	23.03 ± 0.281	11 ± 3.1	nmol/mL	17	
SM d40:2	33.58 ± 0.633	12 ± 2.8	nmol/mL	15	

* Measurement mean ± 1 standard deviation.

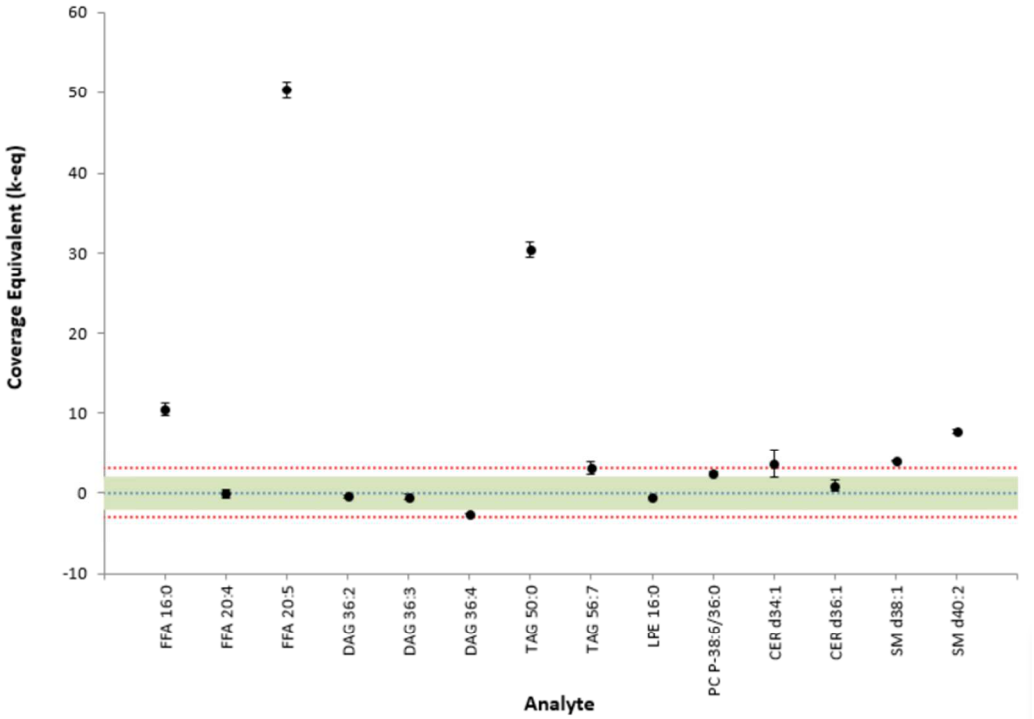
** Consensus mean ± standard uncertainty.

Figure S-2: Example control plot for SRM 1950 - "Metabolites in Frozen Human Plasma". Values are presented as normalized coverage equivalents at the mean (point) and standard deviation (error bars) of measurements, overlaid onto the consensus mean value (blue dotted line). The 95% expanded uncertainty region (green) and 99% expanded uncertainty region (red) for the NIST-ILCE consensus mean estimate value are also displayed. **[A]** Annotated control plot for the data obtained from Bligh–Dyer lipid extracts analyzed by UHPLC-HRMS (Figure S1-A and S2-A). **[B]** Control plot for data obtained from modified Bligh–Dyer lipid extracts analyzed by DI-MS/MS (Figure S1-B and S2-B).

[A]



[B]



TABLES

Table S-1. LipidQC–supported lipid classes and their corresponding abbreviations. The (*) denotes lipid classes with plasmanyl and plasmenyl lipid species for which consensus mean values are provided. Consensus mean values are provided for the plasmanyl and plasmenyl lipid species from the LPC, PC, and PE lipid cases for all materials. LipidQC supports the sum composition (PC 36:0), fatty acid position level annotation "/" (PC 16:0/18:1), and the fatty acid level annotation "_" when the fatty acyl position is unknown (PC 16:0_18:1).

Category	Lipid Class	Abbreviation	Annotation Style
Fatty acyls (FA)	free fatty acids	FFA	FFA 16:0
	eicosanoids		12-HETE
Glycerolipids (GL)	diacylglycerol	DAG	DAG(16:0_18:1)
	triacylglycerol	TAG	TAG(16:0_16:0_18:1)
Glycerophospholipids (GP)	lysophosphatidylcholine	LPC*	LPC 16:0
	phosphatidylcholine	PC*	PC(16:0_18:1)
	lysophosphatidylethanolamine	LPE	LPE 16:0
	phosphatidylethanolamine	PE*	PE(16:0_18:1)
	phosphatidylglycerol	PG	PG(16:0_18:1)
	phosphatidylinositol	PI	PI(16:0_18:1)
	phosphatidylserine	PS	PS(16:0_18:1)
Sphingolipids (SP)	ceramide	CER	CER(d16:0/18:1)
	dihydroceramide	CerOH	CerOH d16:0
	hexosylceramide	HexCer	HexCer(d18:1_18:1)
	lactosylceramide	LacCer	LacCer(d18:1_18:1)
	sphingomyelin	SM	SM(d18:1_18:1)
	sphingosine-1-phosphate	S1P	S1P
	sphinganine-1-phosphate	dhS1P	dhSIP
Sterol lipids (ST)	cholesteryl ester	CE	CE 22:0
	free cholesterol/cholesterol derivatives	FC/CHOL	Cholesterol
	bile acids and derivatives	BA	GCDCA

Table S-2. Number of LipidQC lipid species included for consensus mean values (1) reported by 5 or more laboratories with COD ≤ 40 % or (2) reported by 3 or 4 laboratories with COD ≤ 40 %. The following NIST materials are included in LipidQC: SRM 1950 (Metabolites in Frozen Plasma)

Lipid Class	**SRM 1950**	
	n ≥ 5	*n = 3 or 4*
FFA	5	3
Eicosanoids	3	20
DAG	5	1
TAG	42	7
LPC	25	4
PC	53	4
LPE	6	-
PE	29	2
PG	1	2
PI	13	-
PS	-	2
CER	8	4
CerOH	-	4
HexCer	4	1
LacCer	-	-
SM	30	2
S1P	-	1
dhS1P	-	1
CE	15	3
FC/CHOL	1	1
BA	14	2
Total	254	62