Clinical Lipidomics

CLINICAL LIPIDOMICS AND METABOLISM UNIT (UCA-CCM)

LIPID METABOLISM RESEARCH

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V0.5 – 02/2023

Clinical Lipidomics

VALIDATED METHODOLOGY

Clinical Lipidomics

VALIDATED METHODOLOGY

- ✓Reverse pase LC-MS/MS (SCIEX)
- ✓Targeted approach: more tan 200 lipid species from 19 classes
- ✓Modified Folch extraction for better reproducibility
- ✓3 levels of quality control samples
- ✓Concentration quantitation combining internal standart and matrix calibration.

Abbreviation	Full Name	Lipid Class	Lipid Subclass
ACer	Acylceramide	Sphingolipids	Ceramides
PHCer	Phytoceramide	Sphingolipids	Ceramides
Cer	Ceramide	Sphingolipids	Ceramides
HexCer	Hexosylceramide (Gluco/Galacto)	Sphingolipids	Glycosphingolipids
SM	Sphingomyelin	Sphingolipids	Phosphosphingolipids
DHCer	Dihydroceramide	Sphingolipids	Ceramides
dhHexCer	Dihydrohexosylceramide	Sphingolipids	Glycosphingolipids
dhSM	Dihydrosphingomyelin	Sphingolipids	Phosphosphingolipids
LPC	Lysophosphatidylcholine	Glycerophospholipids	Lysophospholipids
LPE	Lysophosphatidylethanolamine	Glycerophospholipids	Lysophospholipids
PC	Phosphatidylcholine	Glycerophospholipids	Phospholipids
PC_O	Alkyl/Vinil phosphatidylcholine	Glycerophospholipids	Plasmalogens
PE	Phosphatidylethanolamine	Glycerophospholipids	Phospholipids
PE_O	Alkyl/Vinil phosphatidylethanolamine	Glycerophospholipids	Plasmalogens
PI	Phosphatidylinositol	Glycerophospholipids	Phospholipids
SE	Sterol ester	Sterol lipids	Sterol esters
FC	Free cholesterol	Sterol lipids	Free sterols
TAG	Triacylglycerol	Glycerolipids	Neutral glycerolipids
DAG	Diacylglycerol	Glycerolipids	Neutral glycerolipids

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QC EVALUATION

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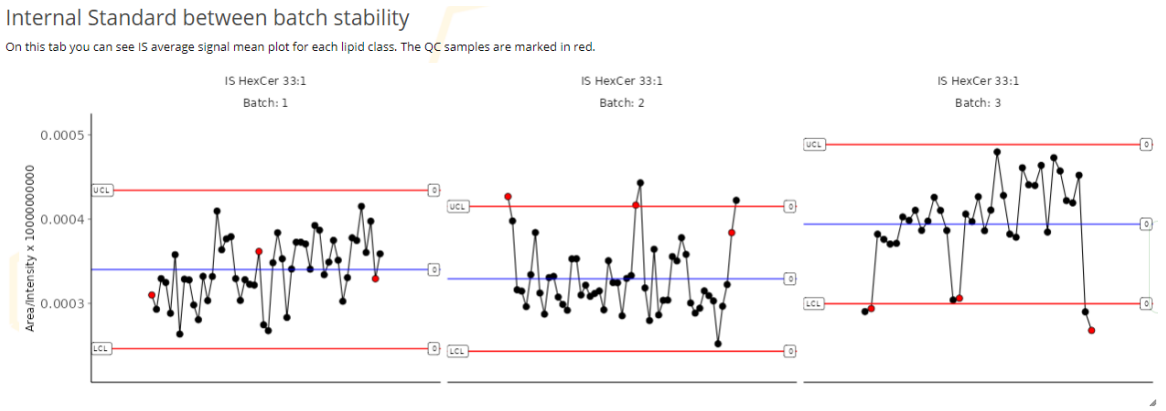
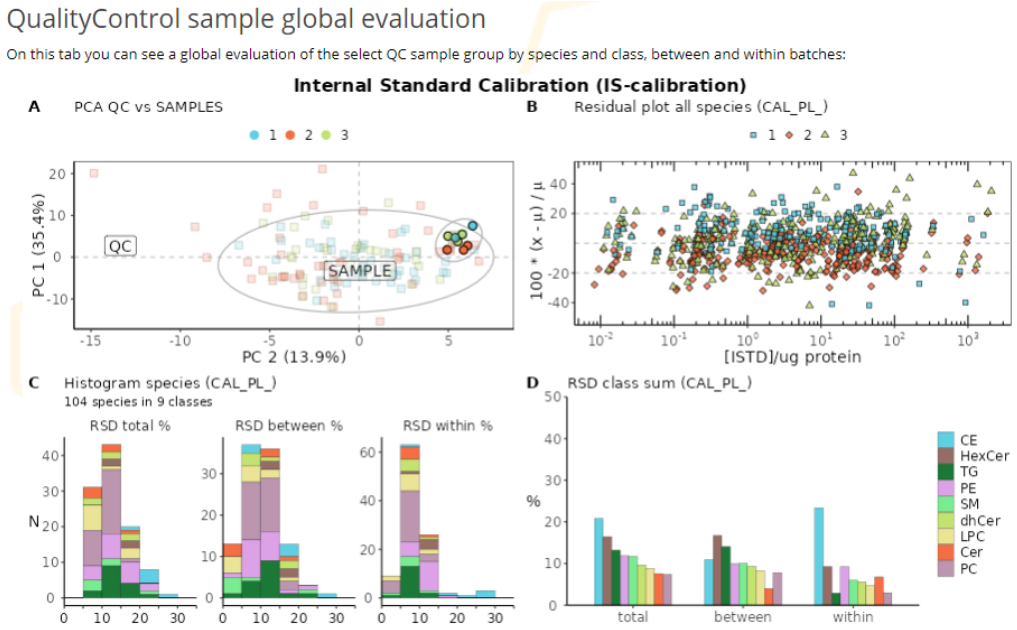
QC EVALUATION

- ✓Extraction evaluation:
  - ✓Internal standart variability →
  - ✓QC intra batch variability →





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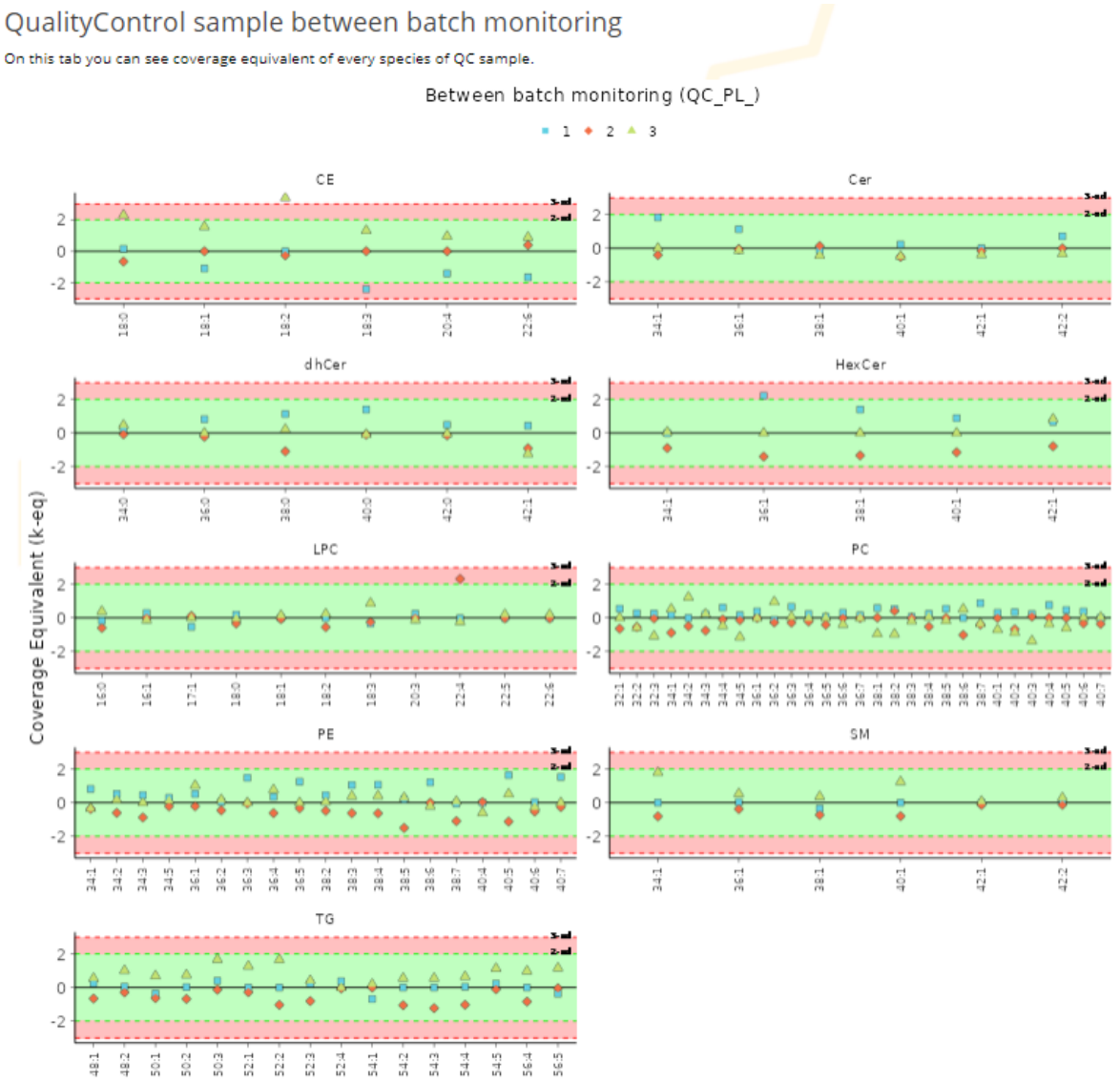
QC EVALUATION

- ✓Robustness evaluation:
  - ✓Long term quantitation accuracy



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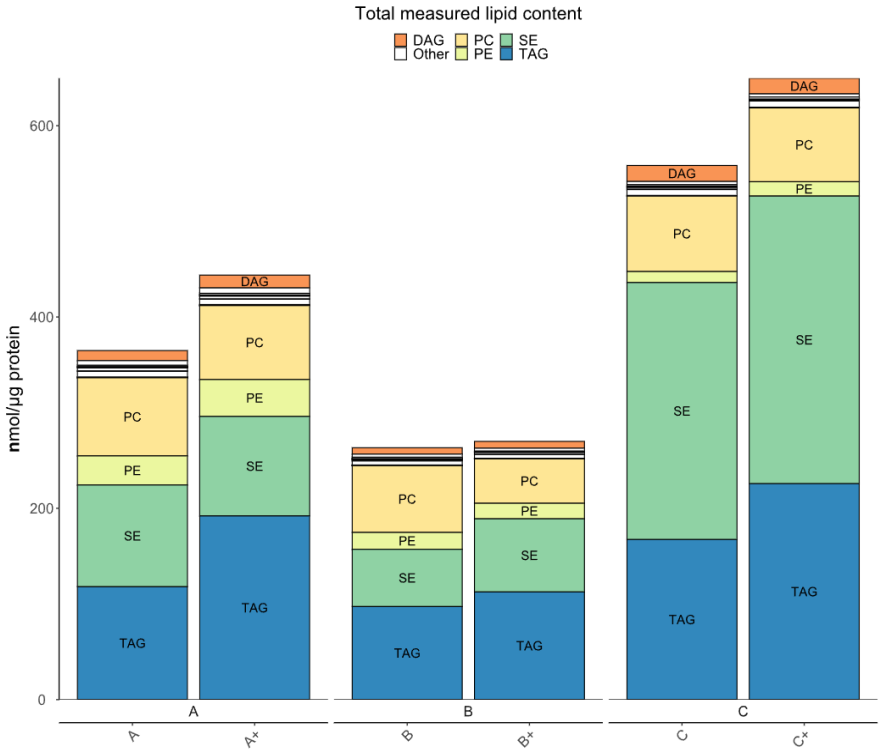
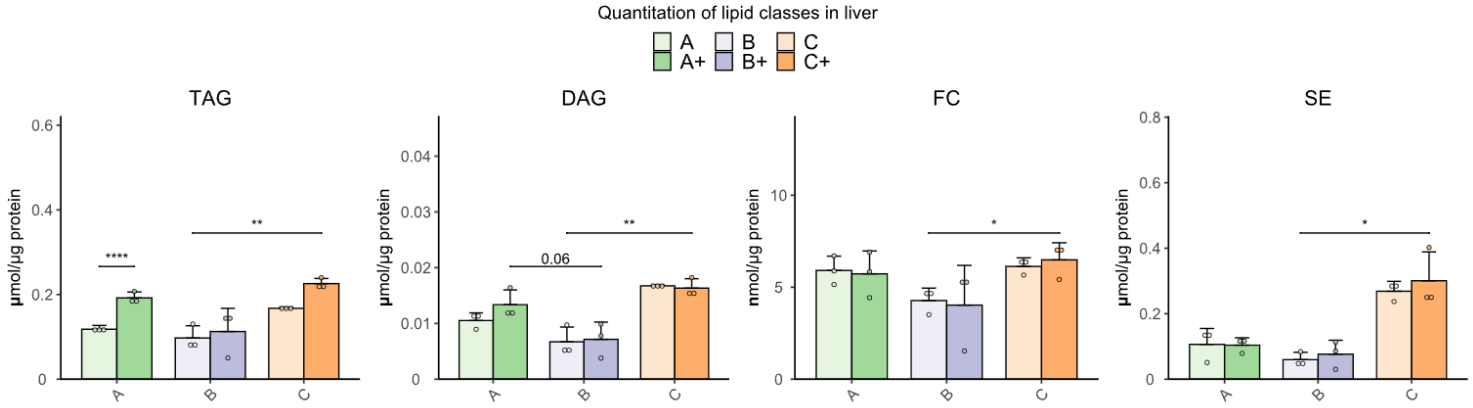
✓Total lipid content



✓Class sum barplot



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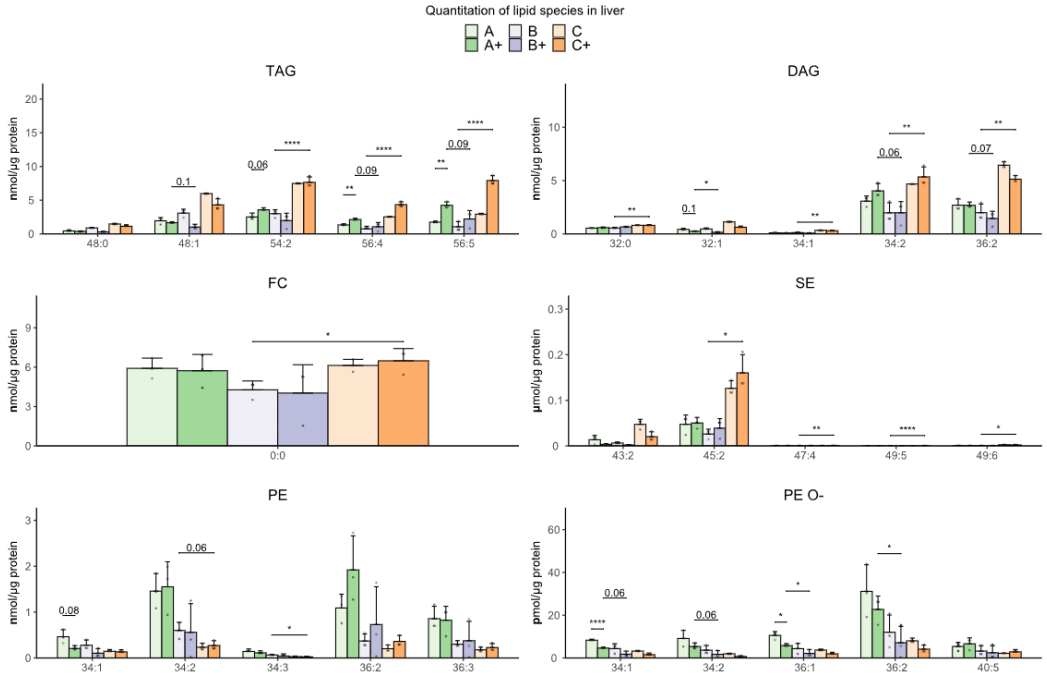
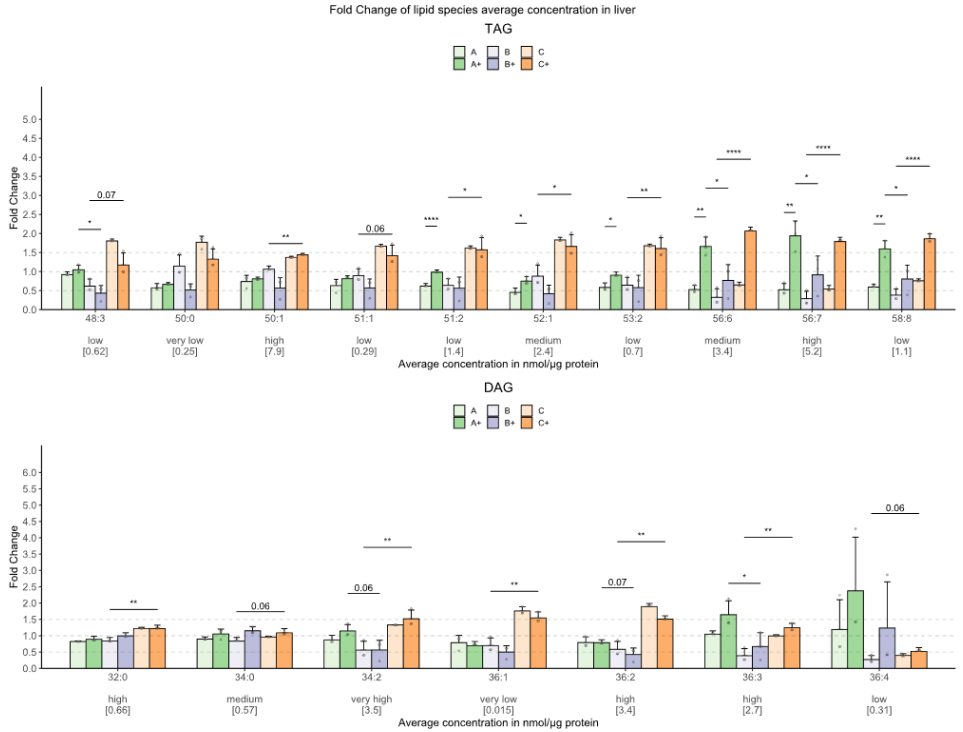
✓Most abundant species barplot



✓Most variable species barplot



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✓Report in Excel format with quantitation data and basic statistics like ANOVA

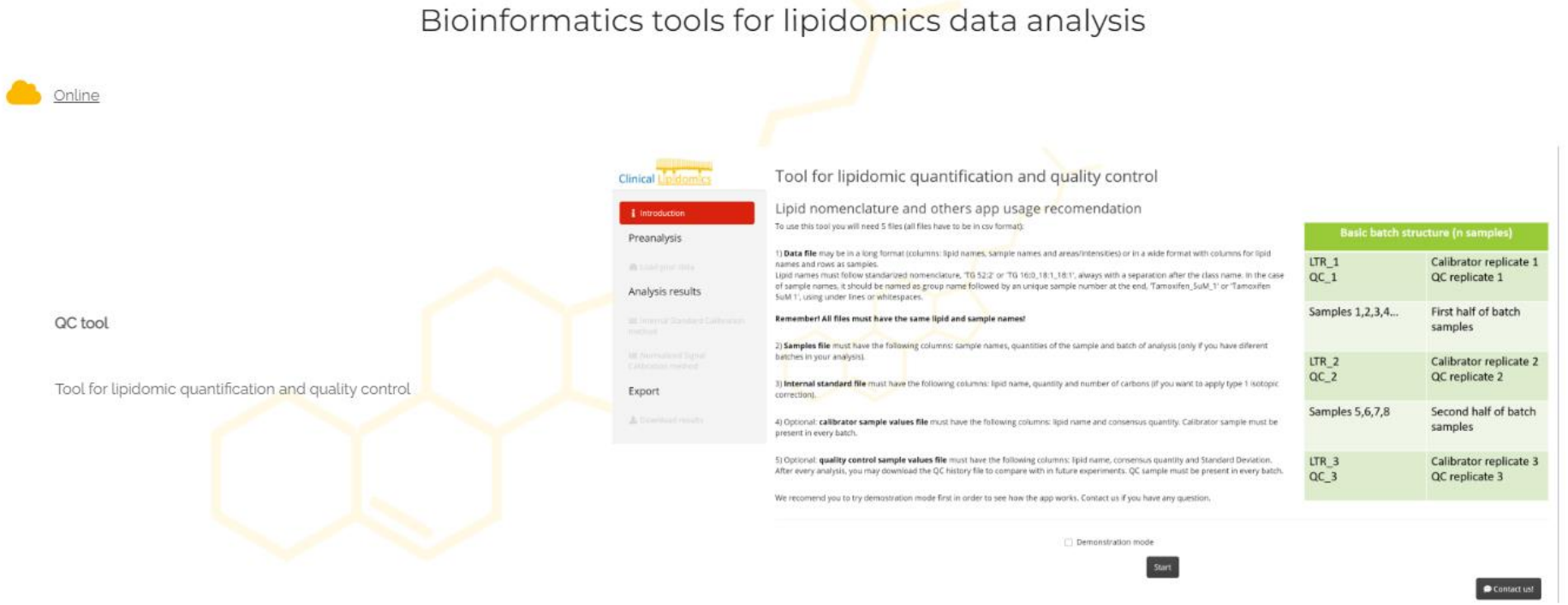
	A	B	C	D	E	F	G	H	I	J	K	L
1	Sample.Nam	GRUPO	ID	Batch.numb	ACer 52:1	ACer 52:2	ACer 54:2	ACer 60:2	ACer 60:3	Cer 32:1	Cer 34:1	Cer 36:1
2	A_1	A	A_1	1	0.00016307	0.00019991	8.2013E-05	0.00180266	0.0019372	0.00037938	0.03117402	0.00319646
3	A_2	A	A_2	1	0.00020158	0.00031084	0.00014775	0.00316138	0.0031085	0.00064981	0.05350234	0.00440434
4	A_3	A	A_3	1	0.00016036	0.00025142	0.00012732	0.00263424	0.00281768	0.00051549	0.0387854	0.00392809
5	A+_1	A+	A+_1	1	0.00037494	0.0003616	0.00017607	0.00366583	0.0036946	0.00053642	0.0561035	0.00669985
6	A+_2	A+	A+_2	1	0.00052449	0.0003915	0.00026374	0.00364553	0.00281404	0.00058012	0.05473476	0.00989767
7	A+_3	A+	A+_3	1	0.00040225	0.00031536	0.00021986	0.00271558	0.00264128	0.00040502	0.03624037	0.00846845
8	B_4	B	B_4	1	0.00011404	0.00016507	0.00010329	0.00152275	0.00159181	0.00042472	0.02220737	0.00244705
9	B_5	B	B_5	1	0.00048811	0.00037204	0.00021925	0.00224716	0.00292582	0.00067522	0.04252782	0.00773245
10	B_6	B	B_6	1	0.0001228	0.00014918	8.9215E-05	0.00143785	0.00156493	0.00031242	0.01644429	0.00208587
11	B+_1	B+	B+_1	1	0.0001641	0.00012267	6.7286E-05	0.00061915	0.00087533	0.00011951	0.00747638	0.00217802
12	B+_2	B+	B+_2	1	0.00029169	0.00027173	0.00014943	0.00226404	0.00270684	0.00034609	0.02081971	0.00536616
13	B+_3	B+	B+_3	1	0.00039475	0.00035448	0.00016784	0.00288894	0.00312693	0.00042206	0.04026197	0.00633812
14	C_7	C	C_7	1	0.00084823	0.0013544	0.0006334	0.00642536	0.00907621	0.00077867	0.03208803	0.0079718
15	C_8	C	C_8	1	0.00125571	0.00159601	0.00077704	0.00805499	0.00986397	0.00094341	0.04974311	0.00994151
16	C_9	C	C_9	1	0.00119996	0.00154768	0.00078864	0.00773286	0.00927252	0.00082468	0.03815688	0.00957301
17	C+_1	C+	C+_1	1	0.00170772	0.00178997	0.00079277	0.00852271	0.00985984	0.00100162	0.06568597	0.01655013
18	C+_2	C+	C+_2	1	0.00144748	0.00141866	0.00062508	0.00680298	0.00827886	0.00084462	0.06033298	0.01392937
19	C+_3	C+	C+_3	1	0.00128673	0.00132886	0.00056014	0.00630602	0.00772406	0.00073918	0.04167854	0.01189657

	A	B	C	D	E	F	G	H	I	J	K	L
1	Lipid.Name	A	A+	B	B+	C	C+	p.signif	A_vs_A+	A_vs_B	A_vs_B+	A_vs_C
2	ACer 52:1	0.00018 ± 0.0	0.00043 ± 0.0	0.00024 ± 0.0	0.00028 ± 0.0	0.0011 ± 0.00	0.00148 ± 0.0	<0.0001	<0.05	ns	ns	<0.05
3	ACer 52:2	0.00025 ± 0.0	0.00036 ± 0.0	0.00023 ± 0.0	0.00025 ± 0.0	0.0015 ± 0.00	0.00151 ± 0.0	<0.0001	ns	ns	ns	<0.001
4	ACer 54:2	0.00012 ± 0.0	0.00022 ± 0.0	0.00014 ± 0.0	0.00013 ± 0.0	0.00073 ± 0.0	0.00066 ± 0.0	<0.0001	<0.05	ns	ns	<0.01
5	ACer 60:2	0.00253 ± 0.0	0.00334 ± 0.0	0.00174 ± 0.0	0.00192 ± 0.0	0.0074 ± 0.00	0.00721 ± 0.0	<0.0001	ns	ns	ns	<0.01
6	ACer 60:3	0.00262 ± 0.0	0.00305 ± 0.0	0.00203 ± 0.0	0.00224 ± 0.0	0.0094 ± 0.00	0.00862 ± 0.0	<0.0001	ns	ns	ns	<0.001
7	Cer 32:1	0.00051 ± 0.0	0.00051 ± 0.0	0.00047 ± 0.0	0.0003 ± 0.00	0.00085 ± 0.0	0.00086 ± 0.0	<0.01	ns	ns	ns	<0.05
8	Cer 34:1	0.04115 ± 0.0	0.04903 ± 0.0	0.02706 ± 0.0	0.02285 ± 0.0	0.04 ± 0.0051	0.0559 ± 0.00	ns	ns	ns	ns	ns
9	Cer 36:1	0.00384 ± 0.0	0.00836 ± 0.0	0.00409 ± 0.0	0.00463 ± 0.0	0.00916 ± 0.0	0.01413 ± 0.0	<0.001	<0.05	ns	ns	<0.01
10	Cer 38:1	0.01731 ± 0.0	0.03085 ± 0.0	0.01415 ± 0.0	0.01868 ± 0.0	0.0212 ± 0.00	0.02223 ± 0.0	ns	<0.05	ns	ns	ns
11	Cer 40:1	0.18002 ± 0.0	0.24983 ± 0.0	0.11712 ± 0.0	0.14804 ± 0.0	0.09426 ± 0.0	0.10252 ± 0.0	<0.05	ns	<0.05	ns	<0.01
12	Cer 41:1	0.0562 ± 0.01	0.05309 ± 0.0	0.03111 ± 0.0	0.03533 ± 0.0	0.04253 ± 0.0	0.05301 ± 0.0	ns	ns	ns	ns	ns
13	Cer 42:1	0.16117 ± 0.0	0.11147 ± 0.0	0.0862 ± 0.00	0.07949 ± 0.0	0.08377 ± 0.0	0.10092 ± 0.0	<0.05	ns	ns	ns	ns
14	Cer 42:2	0.19979 ± 0.0	0.1635 ± 0.02	0.12659 ± 0.0	0.10785 ± 0.0	0.18347 ± 0.0	0.22649 ± 0.0	<0.05	ns	ns	ns	ns
15	Cer 44:1	0.0008 ± 0.00	0.00075 ± 0.0	0.00045 ± 0	0.00042 ± 0.0	0.00057 ± 0.0	0.00091 ± 0.0	ns	ns	ns	ns	ns
16	DAG 32:0	0.54246 ± 0.0	0.58649 ± 0.0	0.5509 ± 0.04	0.65406 ± 0.0	0.80407 ± 0.0	0.80328 ± 0.0	<0.0001	ns	ns	ns	<0.001
17	DAG 32:1	0.42558 ± 0.0	0.25485 ± 0.0	0.46511 ± 0.0	0.15542 ± 0.0	1.12434 ± 0.0	0.62314 ± 0.0	<0.0001	ns	ns	<0.05	<0.01
18	DAG 34:0	0.50941 ± 0.0	0.59905 ± 0.0	0.47488 ± 0.0	0.65413 ± 0.0	0.54618 ± 0.0	0.62044 ± 0.0	<0.05	ns	ns	ns	ns

	A	B	C	D	E	F	G	H	I	J
1	GRUPO	Lipid.Name	N	nmol/ug pro	sd	median	suma	cv	se	ci
2	A	ACer 52:1	3	0.000175	2.3056E-05	0.00016307	0.00052501	13.1745699	1.3311E-05	5.7274E-05
3	A	ACer 52:2	3	0.00025406	5.5514E-05	0.00025142	0.00076218	21.8507264	3.2051E-05	0.0001379
4	A	ACer 54:2	3	0.00011903	3.3642E-05	0.00012732	0.00035708	28.2648419	1.9423E-05	8.3573E-05
5	A	ACer 60:2	3	0.00253276	0.00068502	0.00263424	0.00759829	27.0464774	0.0003955	0.00170169
6	A	ACer 60:3	3	0.00262113	0.00060989	0.00281768	0.00786338	23.268146	0.00035212	0.00151505
7	A	Cer 32:1	3	0.00051489	0.00013522	0.00051549	0.00154468	26.2608329	7.8066E-05	0.00033589
8	A	Cer 34:1	3	0.04115392	0.01135103	0.0387854	0.12346176	27.5818912	0.00655352	0.02819752
9	A	Cer 36:1	3	0.00384296	0.00060842	0.00392809	0.01152888	15.8321672	0.00035127	0.00151141
10	A	Cer 38:1	3	0.01730539	0.0034	0.01715119	0.05191618	19.647059	0.00196299	0.00844607
11	A	Cer 40:1	3	0.18002279	0.01825452	0.17613233	0.54006837	10.1401179	0.01053925	0.04534675
12	A	Cer 41:1	3	0.05620125	0.01828661	0.06187943	0.16860375	32.537737	0.01055778	0.04542647
13	A	Cer 42:1	3	0.16116646	0.03940642	0.17079525	0.48349938	24.4507573	0.02275131	0.09789097
14	A	Cer 42:2	3	0.19978743	0.05125956	0.21049235	0.59936228	25.6570514	0.02959472	0.12733581
15	A	Cer 44:1	3	0.00079765	0.00031086	0.00066035	0.00239294	38.9724785	0.00017948	0.00077223
16	A	DAG 32:0	3	0.54246212	0.01032477	0.54536727	1.62738637	1.90331718	0.00596101	0.02564816
17	A	DAG 32:1	3	0.42557951	0.10099626	0.43723841	1.27673852	23.7314676	0.05831022	0.25088862
18	A	DAG 34:0	3	0.50941449	0.03769659	0.52126027	1.52824348	7.39998337	0.02176414	0.09364352
19	A	DAG 34:1	3	0.10031727	0.02823317	0.1113432	0.3009518	28.1438837	0.01630043	0.07013509
20	A	DAG 34:2	3	3.06940736	0.486431	3.21576112	9.20822209	15.8477174	0.28084107	1.2083616
21	A	DAG 36:1	3	0.01162246	0.00326557	0.01281972	0.03486737	28.097028	0.00188537	0.00811211
22	A	DAG 36:2	3	2.69173776	0.58850726	2.59348551	8.07521329	21.8634692	0.33977482	1.46193307
23	A	DAG 36:3	3	2.81361065	0.26779957	2.74173343	8.44083194	9.51800384	0.15461415	0.66525101
24	A	DAG 36:4	3	0.36639411	0.27977618	0.21569763	1.09918234	76.3593541	0.16152885	0.69500255
25	A	DHCer 34:0	3	0.00152183	0.00044416	0.00142566	0.00456548	29.185691	0.00025643	0.00110334
26	A	DHCer 36:0	3	0.00055977	0.00018099	0.00052591	0.0016793	32.3336789	0.0001045	0.00044961

REPORT FORMAT

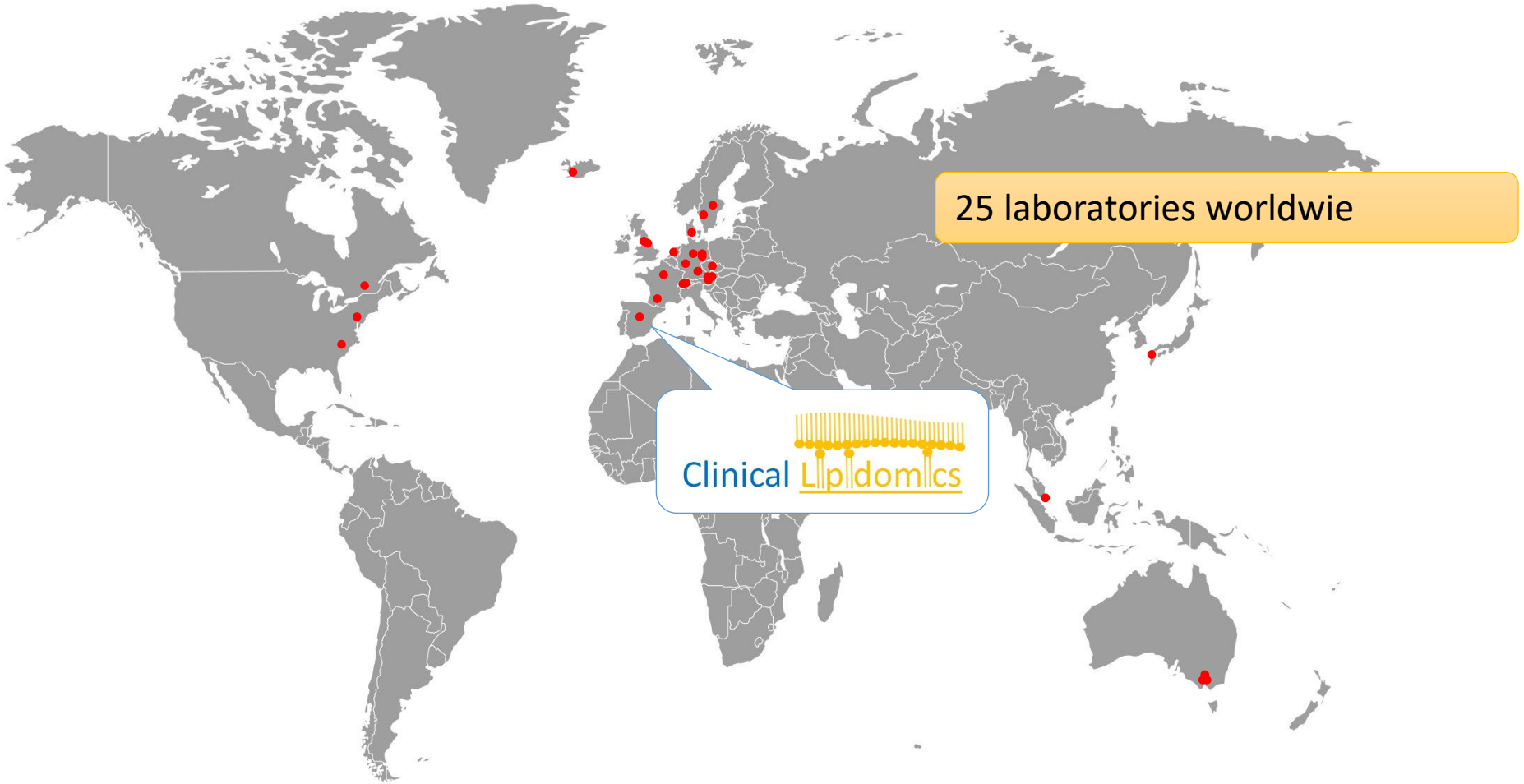
✓Results are compatible with our own QC Tool online tool for lipidomic data mining. Recomended tools can be found here: [Lipidomics tools]



Clinical Lipidomics

OUR NEXT STEPS IN QUALITY IMPROVEMENT

Interlaboratory Trial on Human Plasma Lipidome – International Lipidomic Society



Vvedenskaya, Olga, et al. "Clinical lipidomics–A community-driven roadmap to translate research into clinical applications." Journal of Mass Spectrometry and Advances in the Clinical lab 24 (2022): 1-4.