INTER-LABORATORY REPRODUCIBILITY OF A TARGETED LIPIDOMICS PLATFORM FOR ANALYSIS OF HUMAN SERUM AND PLASMA

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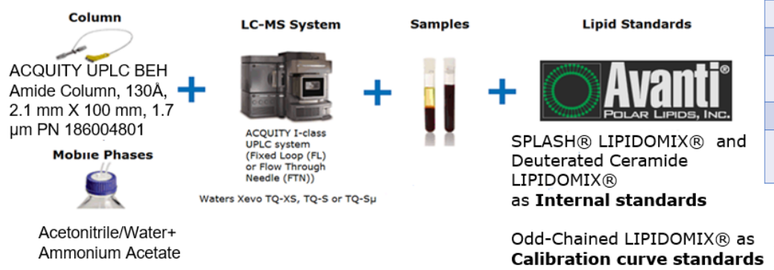
INTRODUCTION

- A general and undisputed dogma of clinical chemistry is that the levels of metabolites circulating in blood plasma are reflective of various aspects of organism homeostasis[1]
- The combination of simplified lipidomic analytical protocols, rapid developments in mass spectrometry technology, and the wide range of potential clinical and biomedical applications suggests a bright future for plasma lipidomics
- Despite the overall success to date, many researchers recognise current community practices make it difficult to harmonize published data and/or make them amenable to multi-omics approaches
- Development is also hindered by lack of communication between research and clinical communities as there is no system in place to assess and cross-correlate plasma lipidomic profiles obtained by different laboratories in various clinical settings
- Furthermore, data is often reported in arbitrary units (ion counts of peak intensity or area) even though quantification of molecule numbers (moles) is necessary for the calculation of the fraction of lipid classes and vital for the detailed interpretation and comparison of large datasets in multi-laboratory studies [2]
- Many in the lipidomics community recognised the need for standardised performance verification parameters and quality control measures for the determination of data quality since batch to batch variations are inherent characteristics of high-throughput analytics

METHODS

Over 2000 lipid species MRMs and a selection of screening method application notes available for download @ www.waters.com/LipidQuan



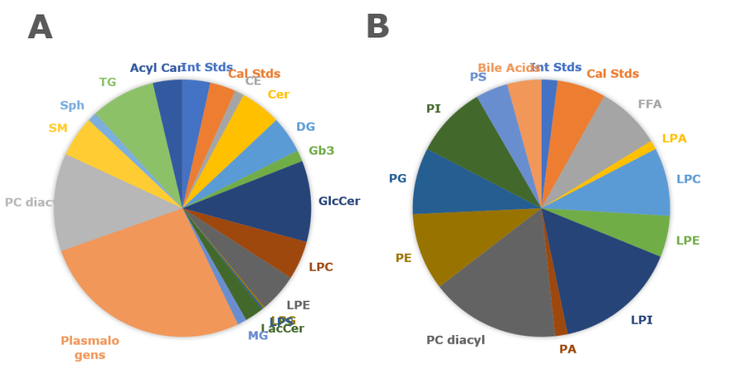


***Figure 2**: LipidQuan instrumentation and LC-MS/MS conditions (Top); **(A)** Lipid species coverage for curated positive mode Plasma Screen; **(B)** Lipid species coverage for negative mode Plasma Screen.*

LIPIDQUAN KEY FEATURES

- Robust and easy to deploy platform, reducing method development and training costs, using Quanpedia™ and dedicated SOPs.
- Rapid LC gradient (8 minute run time), fast data processing and visualization using TargetLynx ™ software and third party infromatics (i.e Skyline, Metaboanalyst for maximum flexibility).
- Improved identication and specificity using MRM transitions based on the fatty acyl chain fragments when applicable as well as the typical head group fragments.
- Sample preparation and data processing can readily be automated.
- Faster and more cost effective than comparable workflows.

	1	2	3	4	5	6	7	8	9	10
A	Double blank	Single blank	Cal 10	Cal 9	Cal 8	Cal 7	Cal 6	Cal 5	Cal 4	Cal 3
B	Cal 2	Cal 1	LLOQQC2 1	LLOQQC2 2	LLOQQC2 3	LLOQQC2 4	LLOQQC2 5	LLOQQC2 6	LLOQQC1 1	LLOQQC1 2
C	LLOQQC1 3	LLOQQC1 4	LLOQQC1 5	LLOQQC1 6	LQC 1	LQC 2	LQC 3	LQC 4	LQC 5	LQC 6
D	MQC 1	MQC 2	MQC 3	MQC 4	MQC 5	MQC 6	HQC 1	HQC 2	HQC 3	HQC 4
E	HQC 5	HQC 6	ULOQQC2 1	ULOQQC2 2	ULOQQC2 3	ULOQQC2 4	ULOQQC2 5	ULOQQC2 6	ULOQQC1 1	ULOQQC1 2
F	ULOQQC1 3	ULOQQC1 4	ULOQQC1 5	ULOQQC1 6	Double blank	Double blank	Double blank	Double blank	Double blank	Double blank
G	Single blank	Single blank	Single blank	MQC 15uL 1	MQC 15uL 2	MQC 15uL 3	MQC 15uL 4	MQC 15uL 5	MQC 15uL 6	DIQC 1 (1:5) 1
H	DIQC 1 (1:5) 2	DIQC 1 (1:5) 3	DIQC 1 (1:5) 4	DIQC 1 (1:5) 5	DIQC 1 (1:5) 6	NIST 1	NIST 2	NIST 3	NIST 4	NIST 5
I	NIST 6	NIST 7	NIST 8	Single blank	NIST(1:4) 1	NIST(1:4) 2	NIST(1:4) 3	NIST(1:4) 4	NIST(1:4) 5	NIST(1:4) 6
J	NIST(1:4) 7	NIST(1:4) 8			Leu Enk 20pg/uL	Leu Enk 20pg/uL		System Suit Sol.	Single blank-Conditioning	Single blank-Conditioning

***Figure 3** Validation test kits (layout shown above) shipped to each test laboratory contained calibration and QC sample extracts prepared following the LipidQuan method guide. System Suitability solutions were included in test kits.*

***Key**: Cal –Calibration Curve, QC– QC samples at 7 concentration levels, MQC 15uL-Minimum sample size assessment, DIQC– Dilution integrity QC, NIST– NIST SRM 1950*

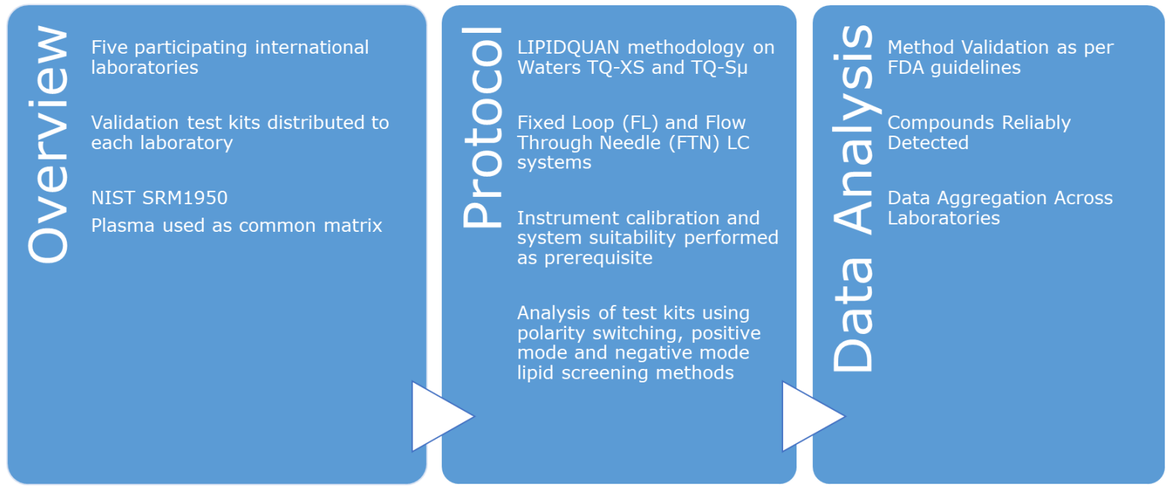


***Figure 1**: LIPIDQUAN platform interlaboratory cross validation study design*

METHOD VALIDATION

Intra day validation assessments of the method were evaluated for various lipid classes using a polarity switching method, positive mode screen (431 MRM transitions) and negative mode screen (446 MRM transitions). A range of analytical attributes were investigated, including linearity, intra- and inter-day accuracy and precision, lower and upper limits of quantification (LLOQ, ULOQ), specificity, carry-over, matrix and other interferences.

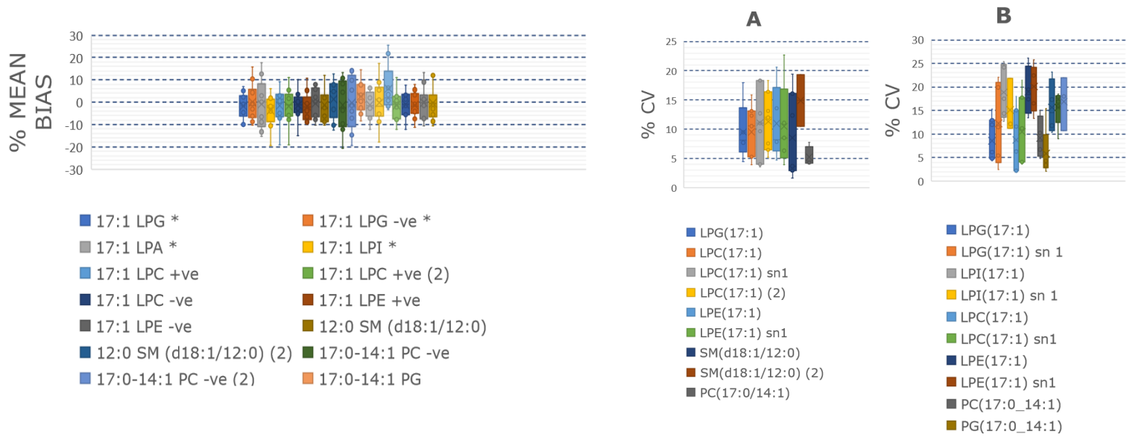
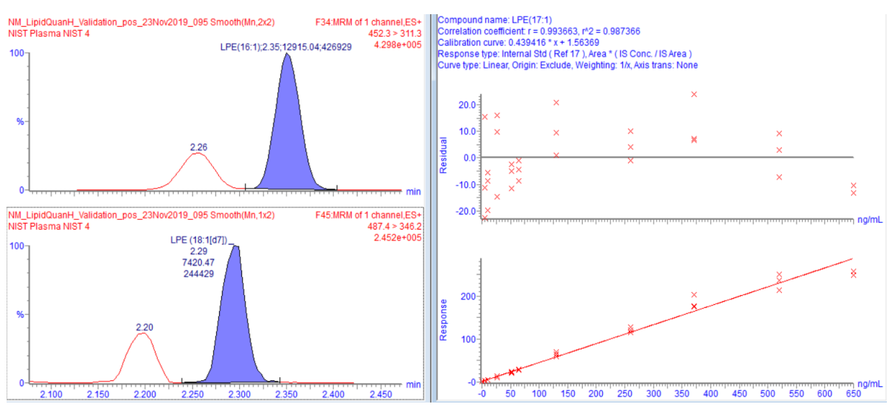


***Figure 4** : Acurracy: Intra-day % mean biases of the calibration standards for the polarity switching method*

***Figure 5** : Precision: Intra day QC positive mode screen method CVs (n=6 for each point on the bar) **(A)** and the negative mode screening method CVs **(B)**.*

QUANTIFICATION OF NIST SRM 1950 USING LIPIDQUAN



	#	ID	Vial	Name	Type	Std. Conc	RT	Area	IS Area	Response	Primary Flags	ng/mL	%Dev	S/N	Sample Text	S/N LOQ	S/N LOD
50	50	NIST 1	2:D,4	NM_LipidQuanH_Validation_pos_23Nov2019_092	Analyte		2.35	12288.517	6759.884	18.179	bb	37.8		39239...	NIST Plasma	YES	YES
51	51	NIST 2	2:D,5	NM_LipidQuanH_Validation_pos_23Nov2019_093	Analyte		2.35	12280.335	7484.612	16.407	bb	33.8		39122...	NIST Plasma	YES	YES
52	52	NIST 3	2:D,6	NM_LipidQuanH_Validation_pos_23Nov2019_094	Analyte		2.35	11046.008	5639.048	19.588	bb	41.0		35507...	NIST Plasma	YES	YES
53	53	NIST 4	2:D,7	NM_LipidQuanH_Validation_pos_23Nov2019_095	Analyte		2.35	12915.042	7428.473	17.405	bb	36.0		276.947	NIST Plasma	YES	YES
54	54	NIST 5	2:D,8	NM_LipidQuanH_Validation_pos_23Nov2019_096	Analyte		2.35	11227.550	6522.446	17.214	bb	35.6		35853...	NIST Plasma	YES	YES
55	55	NIST 6	2:E,1	NM_LipidQuanH_Validation_pos_23Nov2019_097	Analyte		2.35	12288.107	7851.584	15.650	bb	32.1		167.245	NIST Plasma	YES	YES
56	56	NIST 7	2:E,2	NM_LipidQuanH_Validation_pos_23Nov2019_098	Analyte		2.35				bb	36.7		1025.9...	NIST Plasma	YES	YES
57	57	NIST 8	2:E,3	NM_LipidQuanH_Validation_pos_23Nov2019_099	Analyte		2.35				bb	30.6		264.045	NIST Plasma	YES	YES

***Figure 6**: Quantification with TargetLynx™ uses the calibration curve and internal standards to quantify endogenous lipid species of the same class in test samples e.g NIST plasma. In this example, LPE(16:1) in NIST SRM 1950 was quantified based on the response of the LPE 17:1 calibration standards.*

DATA AGGREGRATION

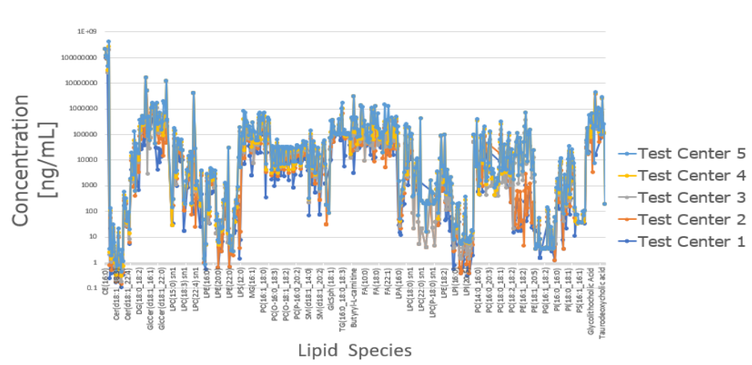


***Figure 7** : Ovelay of average concentrations for lipid species detected in NIST SRM 1950 (n=8) at the participating laborotories, showing good correlation of the results.*

REFERENCES

1. Vvedenskaya, O., Wang, Y., Ackerman, J. M., Knittelfelder, O., & Shevchenko, A. (2019). Analytical challenges in human plasma lipidomics: A winding path towards the truth. TrAC Trends in Analytical Chemistry, 120, 115277. https://doi.org/10.1016/J.TRAC.2018.10.013
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