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Standardized quantitative metabolomics using the biocrates MxP® Quant 500 XL kit across mass spectrometer platforms

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1 Introduction

Robust and reproducible results are the cornerstone of any scientific finding. Such results require standardized and quantitative methods. Over the last decade, biocrates kit technology has emerged as the gold standard in quantitative metabolomics, presenting new possibilities in the study of human health and disease. International ring trials have proven the quality and reproducibility of biocrates kit data[1], allowing researchers to conduct local sample measurements and contribute to the global scientific community with confidence.

A comprehensive ready-to-use and quality-controlled kit for quantitative metabolic profiling has been developed, called MxP Quant 500 XL, targeting 1,019 metabolites across 39 biochemical classes of lipids and small molecules (Figure 1) related to healthy aging, neurodegeneration, and cardiometabolic diseases. Users can get started quickly and easily with the kit, which comes with calibration standards, quality controls, test samples for system checks, consumables, protocols, and quick start guides.

The newly developed WebIDQ workflow manager is a companion cloud software that guides users through the entire workflow and is easily accessible through a web browser. It combines machine learning-based peak picking, automated quantification, and visual validation features to standardize and increase confidence in generated data. The integrated MetaboINDICATOR tool automatically calculates 474 predefined sums and ratios for advanced biological interpretation of enzyme activities and pathophysiological conditions resulting in a total of up to 1493 possible biomarkers.

2 Materials and method

The kit consists of two patented 96-well filter plates with internal standards already integrated, system suitability test samples, lyophilized calibration standards and quality controls (QCs), which were reconstituted according to protocol. Experimental samples, consisting of 11 human plasma samples (5 female, 6 male, age 17-65, absence of medical diagnosis), NIST SRM 1950, and a feces pool from 30 human subjects, were registered in WebIDQ and arranged together with the calibration and QC samples on a 96-well plate layout. All samples except the calibration standards were measured in replicates of three. The worklist was directly exported to the mass spectrometer software and the layout printed for kit preparation. Fecal samples were prepared according to the biocrates protocol for analyzing feces using a Precellys homogenizer and isopropanol as extraction solvent. The kit was prepared according to the user manual with 10 µL of sample pipetted per well on each of the two kit plates followed by derivatization, extraction, and finally dilution into three separate measurement plates: one for LC-MS/MS and two for FIA-MS/MS (Quant 500 and XL part).

Plates were prepared and measured in different laboratories around the globe on different triple quadrupole instruments using optimized LC-MS/MS and FIA-MS/MS

methods. Mass spectrometers evaluated during the study were as follows:
- Agilent 6495C
- SCIEX 5500, 5500+, 6500+
- Waters Xevo TQ-XS

The total run time was 54 hours (80 samples, 2 injections for each of the three measurement plates). Data files were directly processed in WebIDQ with automated quantification, validation, and normalization. Plasma-based quality control samples at different concentration levels were used to automatically assess performance, checking both accuracy and reproducibility. They were also used for data normalization as an essential part of the workflow to correct for any deviations caused by preparation, measurement, or batch effects. Triplicates of plasma samples from healthy human subjects (absence of medical diagnosis), NIST SRM 1950 plasma, biocrates plasma QCs, and feces pool were analyzed by different technicians with individual kits across MS vendor platforms. Quantified data was exported and evaluated in R.

107 small molecules (14 classes)

- Alkaloids (1)
- Amine oxides (1)
- Amino acids (20)
- Amino acid related (30)
- Bile acids (14)
- Biogenic amines (9)
- Carbohydrates and related (1)
- Carboxylic acids (7)
- Cresols (1)
- Fatty acids (12)
- Hormones and related (4)
- Indoles and derivatives (4)
- Nucleobases and related (2)
- Vitamins and cofactors (1)

912 lipids (25 classes)

- Acylcarnitines (40)
- Phosphatidylcholines (78)
- Lysophosphatidylcholines (12)
- Sphingomyelins (15)
- Cholesteryl esters (22)
- Ceramides (29)
- Dihydroceramides (8)
- Hexosylceramides (19)
- Dihexosylceramides (9)
- Trihexosylceramides (6)
- Diglycerides (44)
- Triglycerides (242)
- Phosphatidic acids (41)
- Lysophosphatidic acids (8)
- Phosphatidylethanolamines (95)
- Lysophosphatidylethanolamines (43)
- Phosphatidylglycerols (64)
- Lysophophatidylglycerols (10)
- Phosphatidylinositols (53)
- Lysophosphatidylinositols (16)
- Phosphatidylserines (18)
- Lysophosphatidylserines (12)
- Sphinganines and sphingosines (8)
- Sphinganine and sphingosine phosphates (8)
- Monoglycerides (12)

Figure 1: The MxP® Quant 500 XL kit metabolite panel.

3 Results and discussion

Up to 75% of the 1,019 metabolites were found to be above the limit of detection (LOD) in the analyzed human plasma samples, as well as NIST 1950. Lipid detectability showed coverage up to 85% in human feces and up to 70% in human plasma. The precision was within 20% for all measured matrices for most analytes above LOD depending on the MS platform and the sample matrix. Analyte concentrations were comparable across all tested LC-MS platforms. The automated peak picking feature of WebIDQ simplified peak integration and accelerated metabolite quantification.

Detectability

The average detectability from the 11 human plasma samples (blue bars in Figure 2) showed a distribution of 54% (553 metabolites) to 79% (808 metabolites) across all labs and instruments. The average detectability from the feces pool (red bars in Figure 2) showed a distribution of 56% (570 metabolites) to 81% (828 metabolites), with a significant higher number of detected lipids compared to plasma. The detectability

was defined as the number of metabolites above LOD (out of 1,019) with coefficient of variation (CV) below 30%. The detectability was dependent on the instrument type and condition. Most laboratories showed consistent, high detectability, with only one participant detecting below 600 metabolites. Overall, the detectability across the laboratories was comparable. Table 1 gives the numbers of detected metabolites over the three measurement plates in plasma and feces.

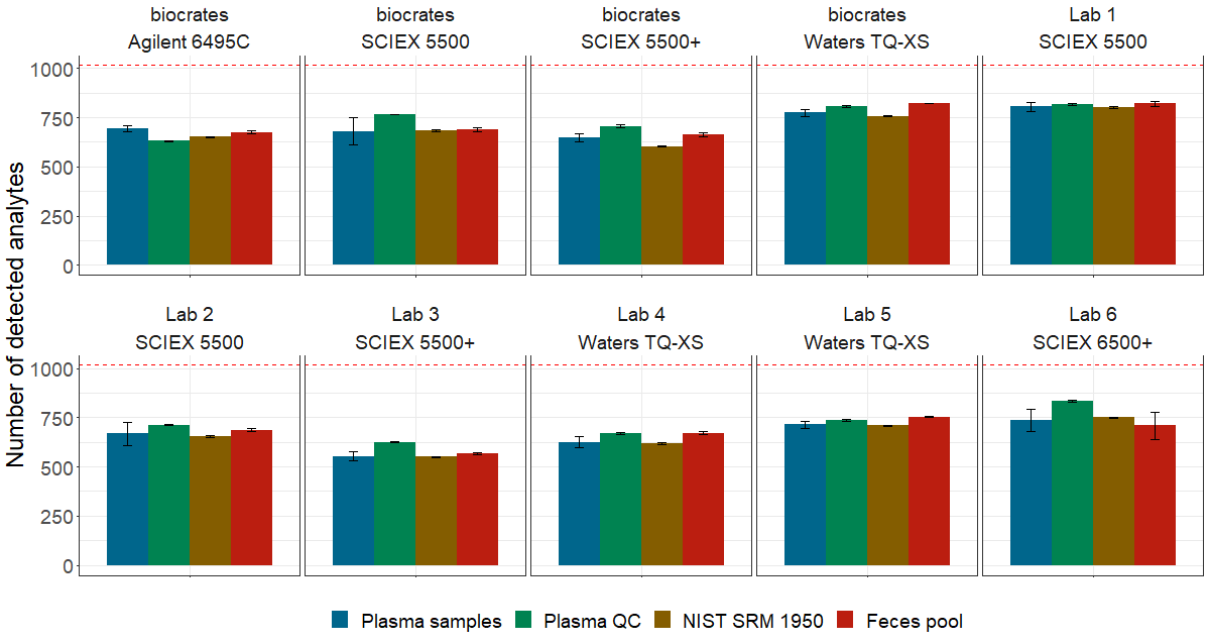

Figure 2: Detectability – Number of metabolites above LOD out of 1,019 and CV<30%.
Note: Lab 3 had higher than expected background signal resulting in fewer analytes above LOD

	bioc Agilent 6495C	bioc SCIEX 5500	bioc SCIEX 5500+	bioc Waters TQ-XS	Lab 1 SCIEX 5500	Lab 2 SCIEX 5500	Lab 3 SCIEX 5500+	Lab 4 Waters TQ-XS	Lab 5 Waters TQ-XS	Lab 6 SCIEX 6500+
Plasma samples (n=11)										
LC	78	81	76	81	74	72	75	69	77	79
FIA	428	381	387	435	424	404	339	398	429	412
XL FIA	183	232	192	258	310	206	139	162	208	259
Total	**689**	**694**	**655**	**774**	**808**	**682**	**553**	**629**	**714**	**750**
Feces pool (n=30)										
LC	95	97	98	104	90	93	94	83	100	99
FIA	301	273	286	369	358	321	235	291	337	286
XL FIA	276	322	282	350	380	271	241	298	315	364
Total	**672**	**692**	**666**	**823**	**828**	**685**	**570**	**672**	**752**	**749**

Table 1: Detectability over the three measurement plates Quant 500 LC, Quant 500 FIA and XL FIA part (number of metabolites above LOD out of 1,019 and CV<30%).
Note: Lab 3 had higher than expected background signal resulting in fewer analytes above LOD.

Intra-laboratory reproducibility

All samples measured in triplicates showed comparable CV distribution across all laboratories and instruments. Plasma samples showed a median CV below 10% for metabolites measured above LOD, the feces pool showed a median CV below 12% (Figure 3 and Table 2). Depending on the sample matrix and the laboratory, 82% to 97% of analytes were below 30% CV and 63% to 92% were below 20% CV.

When considering only the 11 plasma samples from healthy human subjects and comparing the results of the 3 measurement plates (Quant 500 LC, Quant 500 FIA and XL FIA part), the overall %CVs were comparable across all laboratories for metabolites measured above LOD (Figure 4).

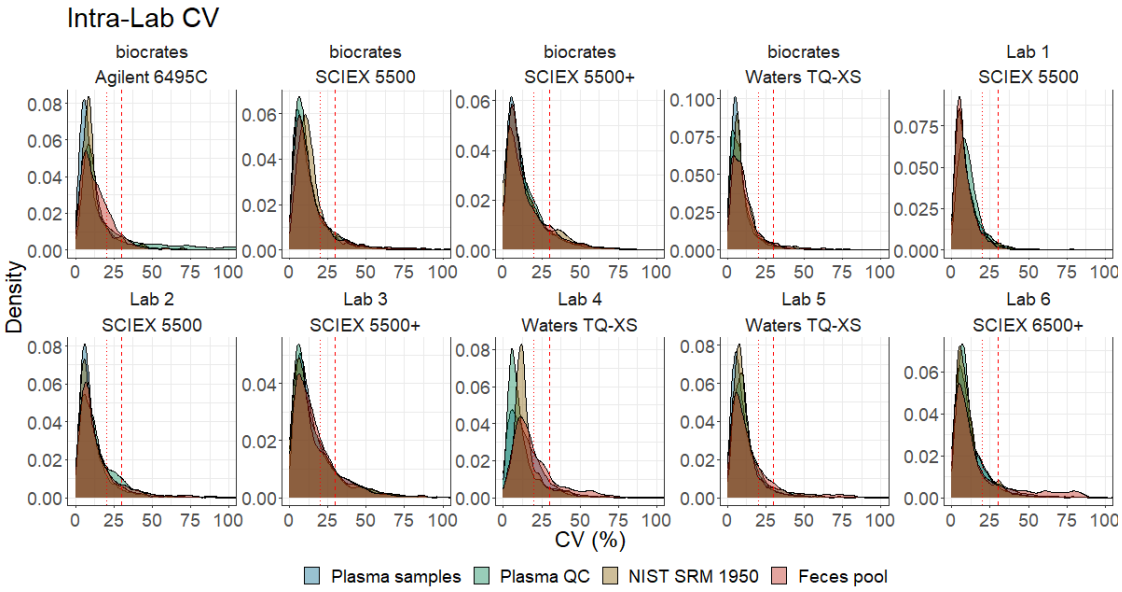

Figure 3: Intra-laboratory CVs of all samples

	bioc Agilent 6495C	bioc SCIEX 5500	bioc SCIEX 5500+	bioc Waters TQ-XS	Lab 1 SCIEX 5500	Lab 2 SCIEX 5500	Lab 3 SCIEX 5500+	Lab 4 Waters TQ-XS	Lab 5 Waters TQ-XS	Lab 6 SCIEX 6500+
Plasma samples (n=11)										
LC	6	6	6	5	6	7	7	9	10	8
FIA	7	12	11	7	8	9	14	12	8	10
XL FIA	13	9	10	7	7	7	13	15	9	9
Average	9	9	9	6	7	8	11	12	9	9
Feces pool (n=30)										
LC	20	8	9	4	5	8	4	11	11	9
FIA	18	14	16	12	9	13	16	18	12	19
XL FIA	11	12	8	9	6	7	12	13	12	5
Average	16	11	11	8	7	9	11	14	12	11

Table 2: Precision (%-CVs) over the three measurement plates Quant 500 LC, Quant 500 FIA and XL FIA part (number of metabolites above LOD)

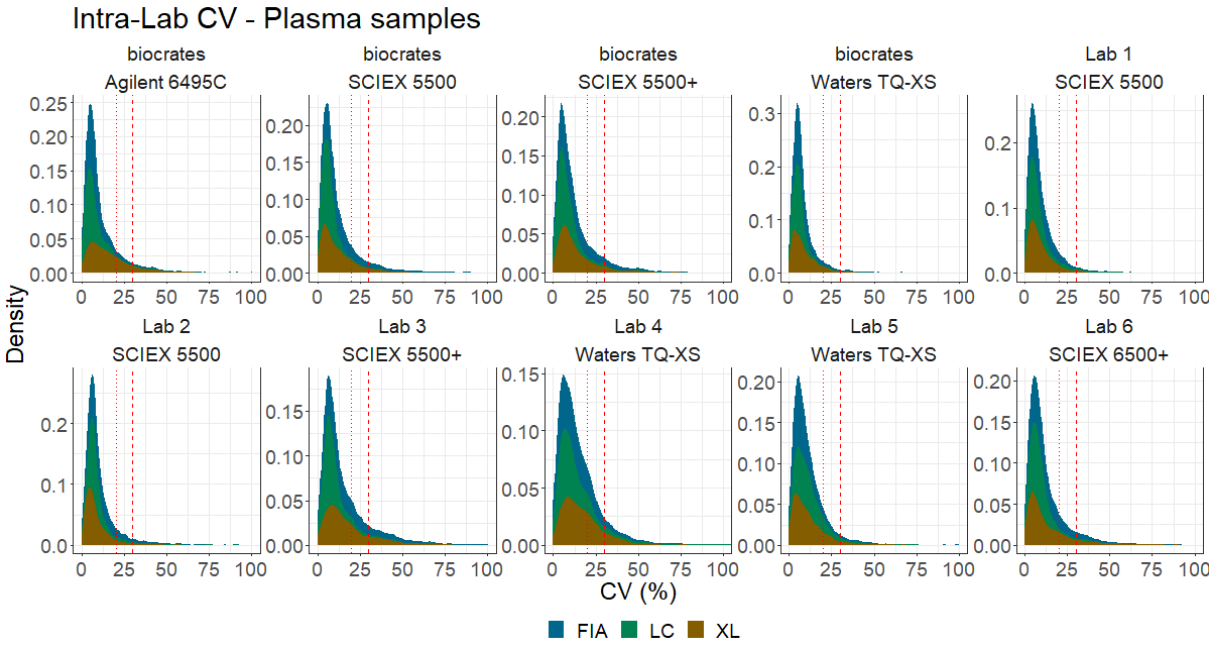

Figure 4: Intra-laboratory CVs of the 11 human plasma samples across the 3 measurement plates (Q500 LC, Q500 FIA and XL FIA part)

Inter-laboratory reproducibility

Inter-laboratory and inter-instrument %CVs were slightly higher compared to the intra-laboratory %CVs, however the majority of the analytes were still below 30% for those measured above LOD (Figure 5)

Inter-laboratory correlation

Figure 6 shows the correlation of concentration values of each instrument compared to Lab 1 (metabolites above LOD and CV below 30%). The results show an excellent inter-instrument correlation.

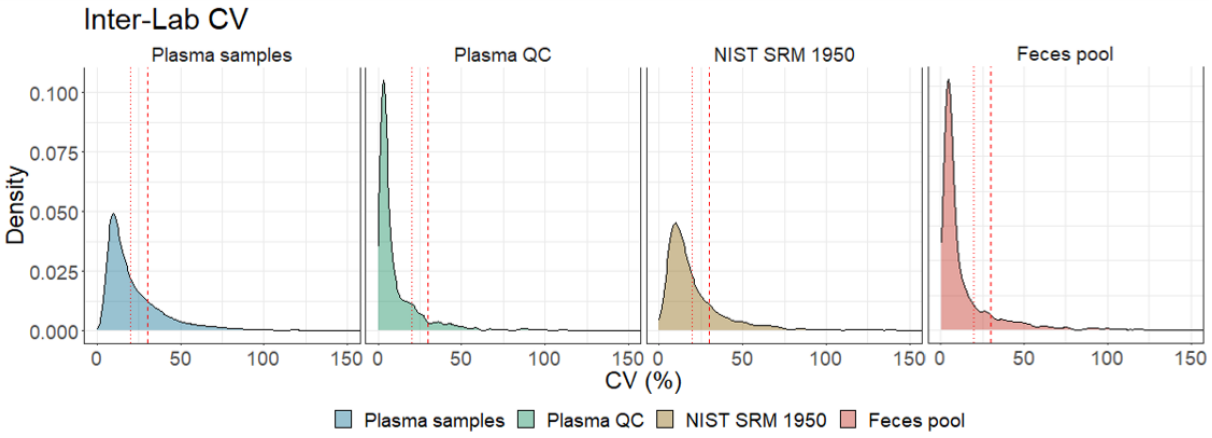

Figure 5: Intra-laboratory CVs of all samples

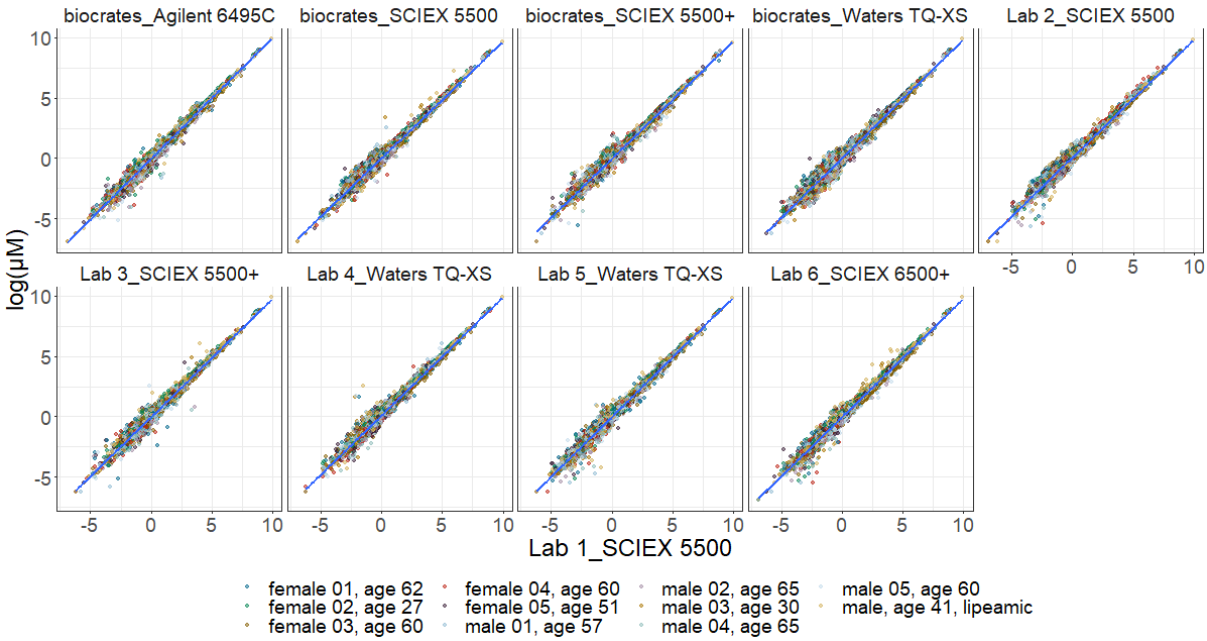

Figure 6: Correlation of plasma sample concentrations from Lab 1 (random representative) compared to concentrations from all other instruments.

4 Conclusions

The results showed high reproducibility and correlation across all laboratories and mass spectrometers used. All instruments displayed good detectably and comparable coverage. It was demonstrated that the MxP® Quant 500 XL kit shows an outstanding analytical performance with excellent lab-to-lab comparability.

5 References

1. Siskos et al. Interlaboratory Reproducibility of a Targeted Metabolomics Platform for Analysis of Human Serum and Plasma. Anal. Chem. 2017; 89(1):656–65.

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