MxP® Quant 1000 kit – Coverage and precision across biological matrices

Franziska Hörburger, Gordian Adam, biocrates life sciences ag, Innsbruck, Austria

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1 Introduction

In metabolomic studies, selecting an appropriate biological matrix is crucial for obtaining robust and meaningful data. Three commonly used matrices — plasma, urine, and feces — offer distinct advantages and considerations that motivate researchers in their choice of sample matrix. MxP® Quant 1000 is a ready-to-use, quality-controlled kit for quantitative metabolomics profiling with mass spectrometry. The metabolite coverage of the kit includes both small molecules and lipids with relevance to microbiome-host metabolism, metabolic health, and chronic disease. MxP® Quant 1000 targets 1,233 analytes across 49 biochemical classes and is validated for use with both human plasma and urine samples.

Plasma is the most widely used matrix for metabolomic analyses for three main reasons: (i) plasma holds a wealth of information on the systemic metabolic state, (ii) blood volume is tightly regulated in the body, providing a convenient matrix for quantitative measurements, and (iii) its collection and processing can be easily standardized across multiple centers, making it a highly valuable matrix for large cohorts and longitudinal studies, and a reliable choice for both fundamental and clinical research.

Urine also offers unique advantages. Its collection is less invasive by nature and well-suited to repeated measurements, facilitating longitudinal studies. One drawback of using urine for quantitative measurement is the high variability of concentrations, which has long been mitigated by measuring creatinine levels to normalize the effect of variations in fluid intake, enhancing the comparability of metabolic profiles across different individuals or time points.

Feces has become a popular matrix for metabolomics, especially in the context of microbiome research, allowing unique insights into how microbiome-produced metabolites influence host health, especially when combined with metabolomics analyses of other host matrices. Although the MxP® Quant 1000 kit was not validated specifically for use in feces, it can be applied to feces using highly standardized sample preparation methods. This technical note also includes information on the precision of the method in this matrix.

Two main factors motivate the choice of a matrix in metabolomics experiments:

- The detectability of metabolites of interest in the matrix
- The analytical performance and reproducibility of the method in the matrix

Other factors such as research objectives, expected applications, and logistical constraints are highly project-specific, however detectability and performance are relevant to all end applications of the MxP® Quant 1000 kit. This information is presented in this technical note to facilitate decision-making in the planning of new metabolomics projects.

2 Methods

Analyses were performed using the MxP® Quant 1000 kit, which includes patented filter plates with integrated internal standards, along with calibration standards and quality controls (QCs). All standards and QCs were reconstituted according to the manufacturer's protocols. All samples along with the calibration and QC samples were registered in the biocrates proprietary WebIDQ workflow manager and organized in a 96-well plate layout. The worklist was generated in WebIDQ, exported directly to the mass spectrometer software, and printed to guide plate preparation.

Plasma, urine, and feces were obtained from healthy individuals (absence of medical diagnosis), aliquoted, and frozen at -80 °C until measurement. The plasma samples were from 11 individuals, urine and fecal samples from 10 individuals. These individuals were not matched between the matrices and had a balanced sex ratio.

In addition to the individual samples, a sample pool was prepared separately for each matrix, combining aliquots of equal volume (equal weight for feces) from every individual. For measurement of plasma and urine, the samples were thawed on ice and used for measurement right away. In preparation for measurement of feces, samples were thawed and homogenized with extraction buffer. The homogenate was centrifuged, and the supernatant was used for analysis. For details on feces sample preparation, please refer to the biocrates application note Metabolome and lipidome analysis of human fecal samples.

In total, 40 µL of sample or sample extract were used for measurement. For each sample, at least three technical replicates were measured.

Each sample underwent derivatization, extraction, and dilution steps where appropriate, as described in the MxP® Quant 1000 kit instructions.

All measurements were carried out on a SCIEX 5500+ triple quadrupole mass spectrometer using optimized acquisition methods. Matrix-specific methods provided with the kit were used for plasma and urine samples. Feces extracts were measured with the protocol for unspecified matrices.

Project management, standardized data processing including AI-driven peak peaking, and metabolite quantification were performed using the WebIDQ workflow manager.

3 Results

3.1 Metabolite detectability

With a total of 1,233 metabolites, the MxP® Quant 1000 kit covers a broad range of small molecules and lipids expected in human plasma, but also in urine and in feces. The panel also includes a few urine- or feces-specific metabolites to provide additional insights for these matrices.

Figure 1 summarizes the detectability of the MxP® Quant 1000 metabolites in healthy human plasma, urine, and feces. Matrix-specific composition and interferences (e.g. suppression effects) can influence the detection profiles among matrices.
In plasma, 1,231 metabolites were targeted using the plasma-specific method. The two remaining metabolites in the panel

(riboflavin (B2) and indolepyruvic acid) are not valid in plasma and were not considered. In plasma, 1,011 metabolites were considered "detectable", i.e., present in concentrations above the limit of detection (> LOD), in at least 50% of the samples. Of these, 927 were detected in at least 80% of the samples. Overall, fewer than 20% of the metabolites were detected in less than 50% of the samples.

In urine, 1,227 metabolites were targeted using the urine-specific method, as six small molecules (N-Ac-Ala, FA 16:0, FA 18:0, FA 20:0, FA 22:0 and bilirubin) are not valid in urine. Of these, 501 were detectable in at least 50% of the samples, with 341 metabolites detected in at least 80% of the samples.
Over 90% of the 726 metabolites that were not detectable in over 50% of the urine samples were lipids, which is in line with the known low abundance of lipids in this matrix.

In feces, 1,226 metabolites were covered using a method for unspecified matrices. Of these, 910 were detectable in at least 50% of the samples, with 788 metabolites detected in at least 80% of the samples. Overall, 316 metabolites were not detectable in over 50% of the samples.

Table 1 lists all metabolite classes covered by the MxP® Quant 1000 kit, the number of metabolites targeted per class, and the number of metabolites detected in at least 50% of the samples for each of the three matrices.

3.2 Matrix-specific precision

The analytical performance of MxP® Quant 1000 kit was assessed for plasma, urine, and feces by calculating the coefficient of variation (CV) of the sample pools (n ≥ 3) for all metabolites that were detected in at least three replicates.

Notably, the CV thresholds are applicable to unnormalized concentrations in the calibrated range. If a metabolite concentration is below the concentration of the lowest or above the concentration of the highest calibrator, the CV may be higher than 30%.

For plasma, 74% of the metabolites were quantified with a CV < 20% (Figure 2). Notably, 49% of all metabolites displayed a CV below 10%, demonstrating the precision of this kit. Of all metabolites detected in plasma, 88% had a CV < 30%. A manual control of the concentrations in those metabolites that did not meet the 30% CV threshold confirmed that almost all of them displayed concentrations below the lower limit of quantification or above the upper limit of quantification, explaining the elevated CVs. **The median CV of metabolites detected in plasma was 10%**.

For urine, 62% of the detected metabolites had a CV < 10%. Of the metabolites detected in urine, 79% had a CV < 20%, while 89% had a CV < 30% (Figure 2). **The median CV of metabolites detected in urine was 6%**.

For feces, 53% of the detected metabolites had a CV < 10%, while 85% had a CV < 20%. Of the metabolites detected in feces, 94% had a CV < 30% (Figure 2). **The median CV of metabolites detected in feces was 9%**.

These results demonstrate high level of precision in fecal samples, even though the kit was not validated specifically for this matrix.

Table 1: Number of MxP® Quant 1000 kit metabolites detected per class in at least 50% of plasma, urine, or feces samples

Metabolite class	No. in class	Plasma	Urine	Feces
Alkaloides	2	1	1	1
Amine oxides	1	1	0	0
Amino acid-related	77	64	71	16
Amino acids	20	20	20	20
Bile acids	24	22	14	23
Biogenic amines	10	7	10	7
Carboxylic acids	8	8	8	7
Catechols	3	0	2	0
Cresols	2	2	2	1
Dicarboxylic acids	25	25	24	9
Fatty acids	39	36	9	30
Hormones	5	4	2	1
Indoles and derivatives	18	10	12	2
Nucleobase-related	14	9	12	2
Nucleobases	5	0	5	4
Nucleotide	2	2	2	0
Organic acids	16	13	12	8
Phenolic acids	22	18	21	11
Phenoxy compounds	2	2	2	0
Polyamines	7	5	6	7
Pyridinecarboxylic acids	6	6	6	2
Sugars	7	7	7	6
Tricarboxylic acids	3	3	3	0
Vitamins and cofactors	9	4	6	2
Acylcarnitines	40	26	31	14
Ceramides	29	24	2	29
Cholesteryl esters	22	21	0	18
Diglycerides	41	20	16	38
Dihydroceramides	8	1	0	8
Dihexosylceramides	9	6	0	8
Trihexosylceramides	6	3	0	5
Hexosylceramides	20	17	2	19
Monoglycerides	12	3	1	11
Lysophosphatidic acids	8	1	7	8
Phosphatidic acids	41	35	14	41
Lysophosphatidylcholines	12	12	3	10
Phosphatidylcholines	76	73	23	46
Lysophosphatidylethanolamines	43	23	23	43
Phosphatidylethanolamines	95	83	47	91
Lysophosphatidylglycerols	10	8	1	10
Phosphatidylglycerols	64	62	16	64
Lysophosphatidylinositols	15	13	3	15
Phosphatidylinositols	53	53	8	52
Lysophosphatidylserines	12	2	8	12
Phosphatidylserines	18	15	11	18
Sphinganines and sphingosines	8	4	7	8
Sphinganine and sphingosine phosphates	8	3	3	8
Sphingomyelins	14	14	5	13
Triglycerides	242	219	4	134
Total	1,233	1,011	501	910

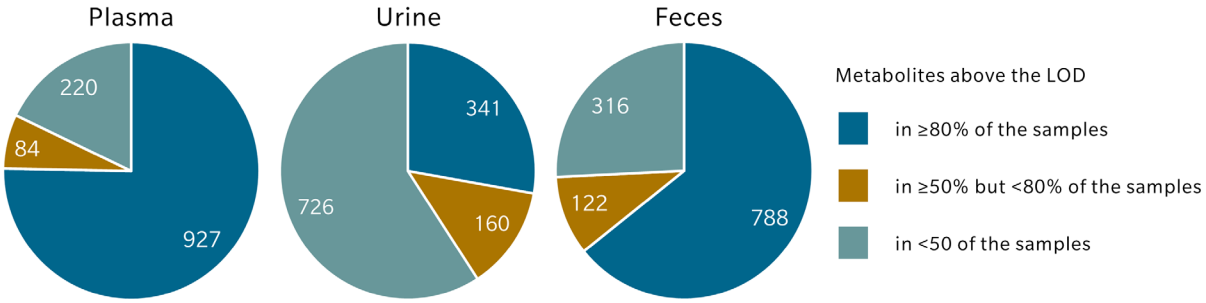

Figure 1: Overview of metabolite detectability in plasma, urine, and feces using the MxP® Quant 1000 kit

4 Conclusions

The MxP® Quant 1000 kit offers extremely broad metabolite coverage and exceptional performance across plasma, urine, and feces with some subtle differences that are captured in matrix-specific methods.

The vast majority of the metabolites targeted with the kit were quantified in plasma samples, with high detectability for both small molecules and lipids. In plasma, the overall performance was excellent with a **median CV of 10%**.

Detection in urine was also robust for the metabolite classes expected in this matrix. Despite lipid concentrations being near LOD in urine, the **median CV of 6%** demonstrates the impressive analytical performance of the kit for this matrix.

Feces is an inhomogeneous matrix with high biological variance. The standardized sample preparation and high analytical performance of the MxP® Quant 1000 kit achieved a precision comparable to plasma, with a slightly smaller metabolite coverage.

Datasets obtained with the MxP® Quant 1000 kit can be even further expanded with up to 648 sums and ratios of metabolites calculated with the MetaboINDICATOR tool of the WebIDQ workflow manager. These indicators were chosen for their relevance to biomedical research and contribute to a total of 1,881 potential biomarkers covered by the MxP® Quant 1000 kit, setting a new standard of excellence in quantitative metabolomics.

In practice, the MxP® Quant 1000 kit is well-suited for all three matrices regarding coverage and analytical performance. It is the only validated targeted metabolomics kit commercially available with this broad coverage for plasma, urine and feces analysis. Internal measurements have shown that the kit also performs well for other non-validated matrices.

This matrix versatility greatly expands the application of the MxP® Quant 1000 kit, enabling cross-matrix comparative studies relevant to microbiome-host interactions, the gut-liver axis, and metabolite excretion. The possibility to follow pathways across biological compartments also opens doors for implementation in systems biology approaches, with a method perfectly suited to multiomics integration.

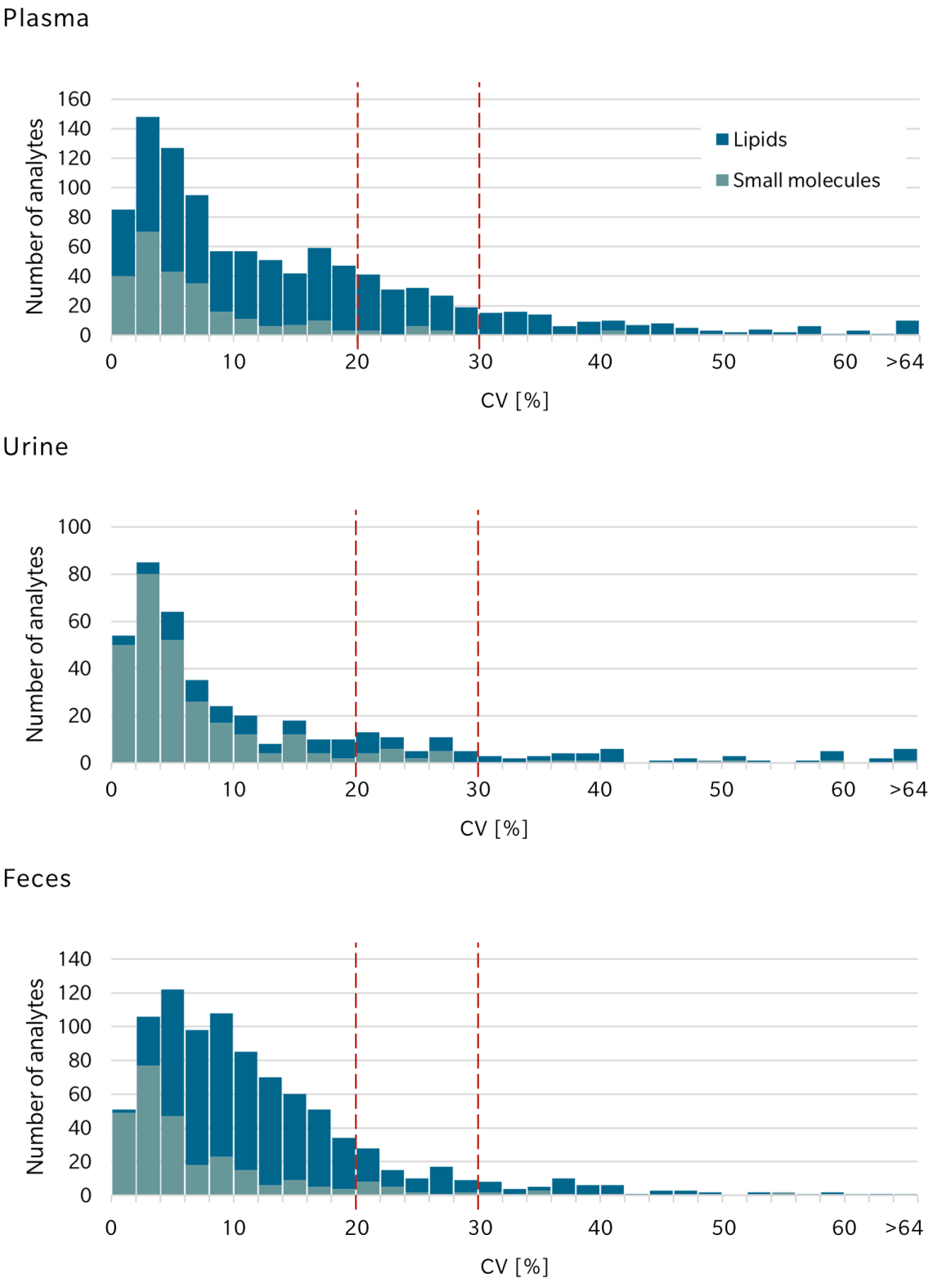

Figure 2: Histogram of all metabolite CVs calculated from concentrations of pooled plasma (n=3), urine (n=3), and fecal (n≥3) samples. The CV thresholds of 20% and 30% as described in the text are given as red dashed line