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Comparative analysis of the MxP® Quant 500 XL and MxP® Quant 1000 kits

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1 Introduction

Standardization, reproducibility, and comparability are at the heart of biocrates' targeted metabolomics solutions. The MxP® Quant 500 XL kit set a benchmark in 2023 with the ability to quantify over 1,000 metabolites across a wide range of biochemical classes, providing scientists with one of the most comprehensive standardized assays available. With the launch of the MxP® Quant 1000 kit in 2025, biocrates has taken the next step in expanding coverage to over 1,200 metabolites, offering researchers an even deeper view into human health and disease metabolism with the same trusted robustness and reproducibility. The kit is also available in a modular format, with the small molecule panel offered as the MxQuant kit and the lipid panel as the LxQuant kit, providing additional flexibility depending on research needs.

A key consideration for laboratories transitioning to the new kit is the continuity of data across studies and time. To demonstrate this, a set of 12 human plasma samples was analyzed using both kit generations –the MxP® Quant 500 XL kit in 2023 and the MxP® Quant 1000 kit in 2025. This technical note highlights the comparability of results, correlation between kits, and reproducibility of measurements. While differences in assay chemistry and plate design can influence the comparability of certain metabolite classes, overall results confirm that researchers can build confidently on existing datasets while benefiting from the expanded metabolite coverage of the MxP® Quant 1000 kit.

2 Materials and method

Two generations of biocrates' targeted metabolomics kits were applied:

MxP® Quant 500 XL kit (2023)

- Utilizes phenylisothiocyanate (PITC) derivatization with pyridine reagent for small molecule analysis.
- Certain lipid classes are measured on the PITC plate, even though they are not chemically derivatized. The presence of PITC reagent, however, may have an influence on these analytes.
- Additional lipid classes are covered by a separate XL plate (underivatized).

MxP® Quant 1000 kit (2025)

- Utilizes separate PITC derivatization with triethylamine reagent and 3-nitrophenylhydrazine (3-NPH) derivatization plates, enabling the quantification of new small-molecule classes and improving coverage for several existing classes.
- Some metabolite classes originally measured on the PITC plate in the MxP® Quant 500 XL kit were transitioned to the 3-NPH plate in the MxP® Quant 1000 kit.
- All lipid classes are consolidated on a dedicated lipid plate, where no derivatization reagent is applied, reducing the risk of reagent effects on lipid measurements.

The kits consist of patented 96-well filter plates, system suitability test mixtures, calibration standards, internal standards and quality controls (QCs).
The same set of samples, consisting of 11 human plasma samples (5 female, 6 male, age 17-65, absence of medical diagnosis) and NIST SRM 1950, was analyzed in with MxP® Quant 500 XL in 2023 and with MxP® Quant 1000 in 2025. Samples 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. The kit was prepared according to the user manual. Small molecules were analyzed using optimized LC-MS/MS methods. Lipids were analyzed using optimized FIA-MS/MS methods. All measurements were performed on a Waters Xevo TQ-XS mass spectrometer coupled to the Waters ACQUITY UPLC I-Class System. Data files were directly processed in the WebIDQ workflow manager with automated AI-driven peak picking, quantification, validation, and normalization. Plasma-based quality control samples at different concentration levels were used to automatically assess performance, checking both accuracy and reproducibility. The quantified data was exported to R for plotting and evaluating analytical performance.

3 Results and discussion

Detectability

Detectability was evaluated in the 12 human plasma samples (Figure 1), including NIST SRM 1950. For the 906 lipids quantified in both kits, detectability was comparable at ~83%. For the 107 small molecules common to both kits, the MxP® Quant 1000 kit showed slightly higher detectability compared to the MxP® Quant 500 XL (85% vs. 78%). Detectability was defined as the proportion of metabolites measured above the limit of detection (LOD) in at least one of the replicates. While differences in instrument conditions across the two measurement years may also have influenced the results, the data indicate that the MxP® Quant 1000 kit provides at least comparable, and in some cases, improved detectability.

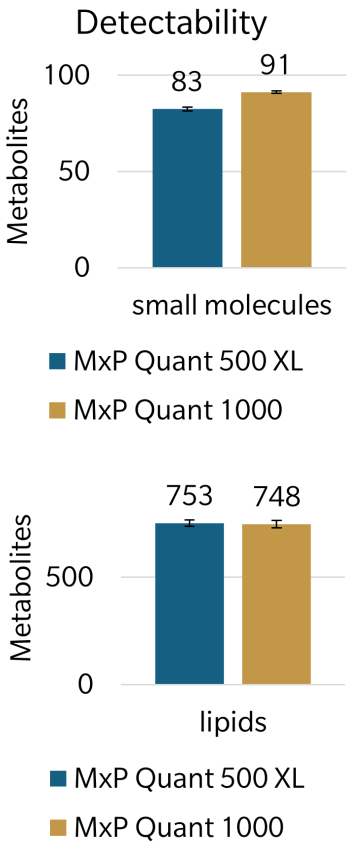

Figure 1: Detectability out of 107 small molecules (above) and out of 906 lipids (below) covered in both kits

Comparability

Overall, the majority of metabolites showed good agreement between the MxP® Quant 500 XL and MxP® Quant 1000 kits. Correlation across shared analytes was generally high, confirming consistency of measurement between kit generations. Most analytes demonstrated very good correlation, with regression factors above 0.9 in both the small molecule and lipid panels (Figure 2). A larger proportion of lipid analytes fell below this threshold compared to small molecules, which may be partly explained by the presence of PITC reagent in the MxP® Quant 500 XL kit, where most lipids were analyzed on the PITC plate despite not being derivatized. In contrast, the MxP® Quant 1000 kit quantifies all lipids on a dedicated lipid plate without PITC.

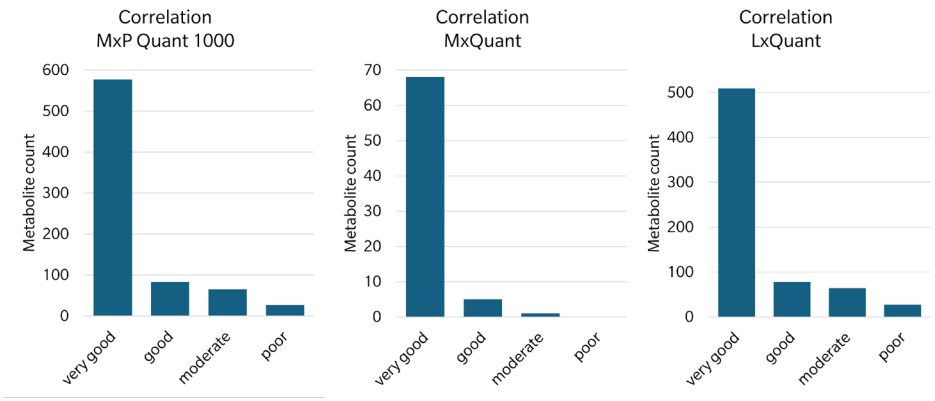

Figure 2: Correlation of the MxP® Quant 500 XL metabolite concentrations above the limit of detection (LOD) to MxP® Quant 1000 (total panel), MxQuant (small molecules only) and LxQuant (lipids only)

While the majority of small molecules showed robust comparability, a subset displayed notable differences in quantified concentrations, as highlighted in the heatmap (Figure 3). Closer inspection revealed that these discrepancies can largely be explained by methodological and technical differences between the kits.

Calibration strategy

Many shared metabolites are quantified using different calibration approaches across the two kits. For instance, HArg, ProBetaine, TrpBetaine, TLCA, AconAcid, HipAcid, Lac, FA 20:2, FA 22:6, and Xanthine are quantified in the MxP® Quant 500 XL kit using only one-point calibration against their internal standards. In contrast, the MxP® Quant 1000 kit applies a full 7-point calibration curve, combining internal standards with external calibration standards. This leads to higher quantification accuracy, but also shifts in absolute concentration values. Overall, the MxP® Quant 1000 kit incorporates substantially more calibration standards than its predecessor, enabling a higher level of analytical robustness.

Derivatization chemistry

The MxP® Quant 500 XL kit relies exclusively on PITC derivatization for small molecules, whereas the MxP® Quant 1000 kit introduces 3-NPH derivatization for specific metabolite classes. This affects analytes such as cystine, lactate, and aconitic acid, which are measured on the PITC plate in MxP® Quant 500 XL but analyzed with 3-NPH chemistry in the MxP® Quant 1000, resulting in different retention times and response factors. In addition, fatty acids, which were not derivatized on the PITC plate in MxP® Quant 500 XL, are now derivatized with 3-NPH in the MxP® Quant 1000.

Internal standard assignment

Internal standard mapping also contributes to differences. For example, cystine is normalized against a methionine internal standard in MxP® Quant 500 XL, but against its corresponding internal standard in MxP® Quant 1000. In general, the MxP® Quant 1000 kit employs substantially more internal standards than the MxP® Quant 500 XL, enabling a higher level of analytical normalization, precision and accuracy in quantification

Chromatographic differences

Some PITC-derivatized metabolites show divergences in retention times and peak shapes that also explain shifts in certain analytes. These shifts are mainly caused using triethylamine reagent in MxP® Quant 1000 instead of pyridine as used in MxP® Quant 500 XL. For HArg, the MxP® Quant 500 XL measurements show a separated

pre-peak, while in the MxP® Quant 1000 kit only a single clean peak is observed. For ProBetaine, samples measured with MxP® Quant 500 XL show much higher peak intensities relative to MxP® Quant 1000. Differences in column setup, sample preparation and derivatization reagent may add to this divergence.

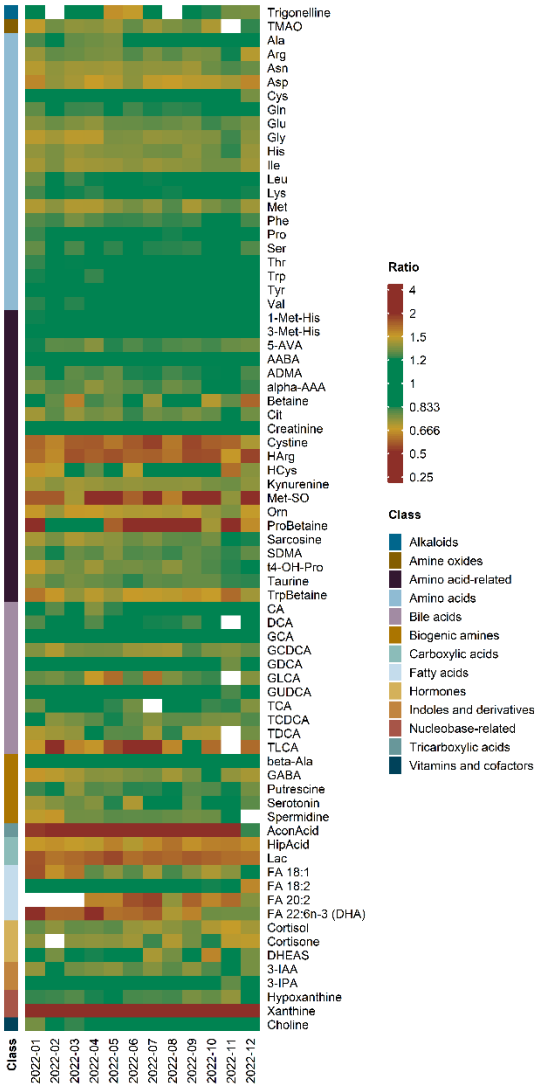

Figure 3: The heatmap shows the concentration differences of small molecules of the same samples measured with MxP® Quant 1000 vs. MxP® Quant 500 XL. Only small molecules > LOD in at least 75% of the samples are shown. Green: ratio of 1 with ≤ 1.2-fold change between the two kits. Yellow: 1.5-fold change, Red: ≥ 2-fold change

Observed concentration differences in lipids can be explained in part by the presence or absence of PITC and pyridine reagents, and in part by the use of different internal standards for calibration. As shown in Figure 4, these factors contribute to systematic shifts in measured concentrations at the level of entire lipid classes. When analyzed with MxP® Quant 1000, cholesteryl esters, sphinganines and sphingosines, and phosphatidylserines consistently show higher concentrations than with the MxP® Quant 500 XL, whereas dihydroceramides and lysophosphatidyl-serines are consistently lower. For the remaining 20 lipid classes, the average ratio between the two kits per class does not exceed a 1.3-fold change. Nevertheless, individual metabolites within these classes can still display marked differences between kits.

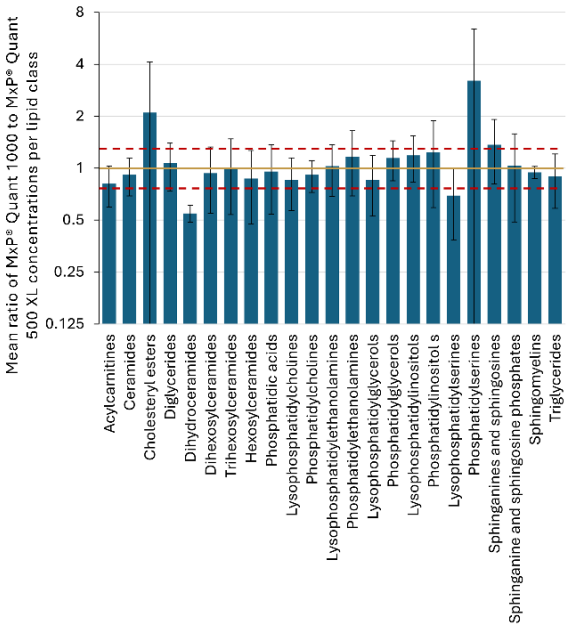

Figure 4: Mean ratio of MxP® Quant 1000 to MxP® Quant 500 XL concentrations, summarized over all samples and all metabolites per lipid class. Ochre line marks a ratio of 1, dashed red lines mark a 1.3-fold change threshold

Taken together, these examples illustrate that while overall comparability between the two kit generations is high, improvements in calibration strategy, derivatization chemistry and internal standard assignment account for the discrepancies observed in a limited number of analytes. Users can therefore expect continuity in the majority of results, while being aware that methodological refinements in the MxP® Quant 1000 kit may lead to more accurate but not always directly comparable values for specific metabolites.

Reproducibility

Reproducibility was assessed by calculating coefficients of variation (CVs) for metabolites above the LOD. All samples measured in triplicate showed comparable CV distributions across sample types (Figure 5). With the MxP® Quant 500 XL kit, mean CVs were 7% for small molecules and 11% for lipids across all sample types. The MxP® Quant 1000 kit showed slightly improved reproducibility, with mean CVs of 4% for small molecules and 8% for lipids. Although instrument conditions across different measurement years may have contributed to this difference, the results indicate that the MxP® Quant 1000 kit delivers at least comparable or potentially improved reproducibility.

Accuracy

Accuracy was evaluated using NIST SRM 1950 plasma for a panel of amino acids and selected metabolites (Figure 6). Both the MxP® Quant 500 XL and MxP® Quant 1000 kits showed results within the commonly accepted ±20% range for the majority of analytes, with MxP® Quant 1000 showing more consistent centering around 100% for the most of the metabolites.

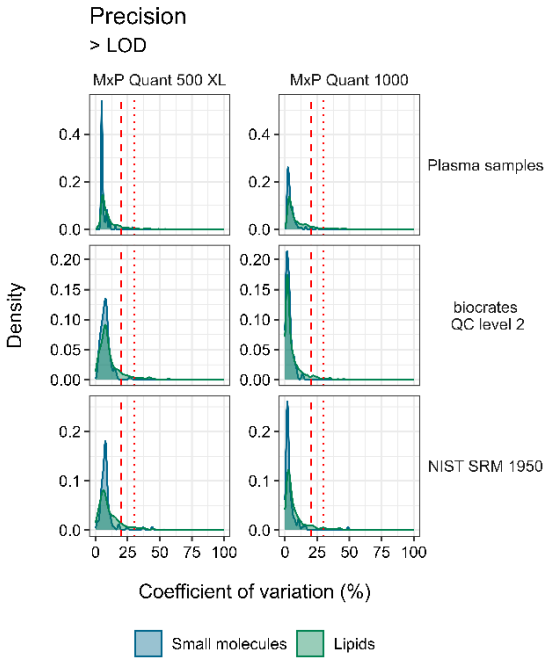

Figure 5: Coefficients of variation (CVs) of all sample types

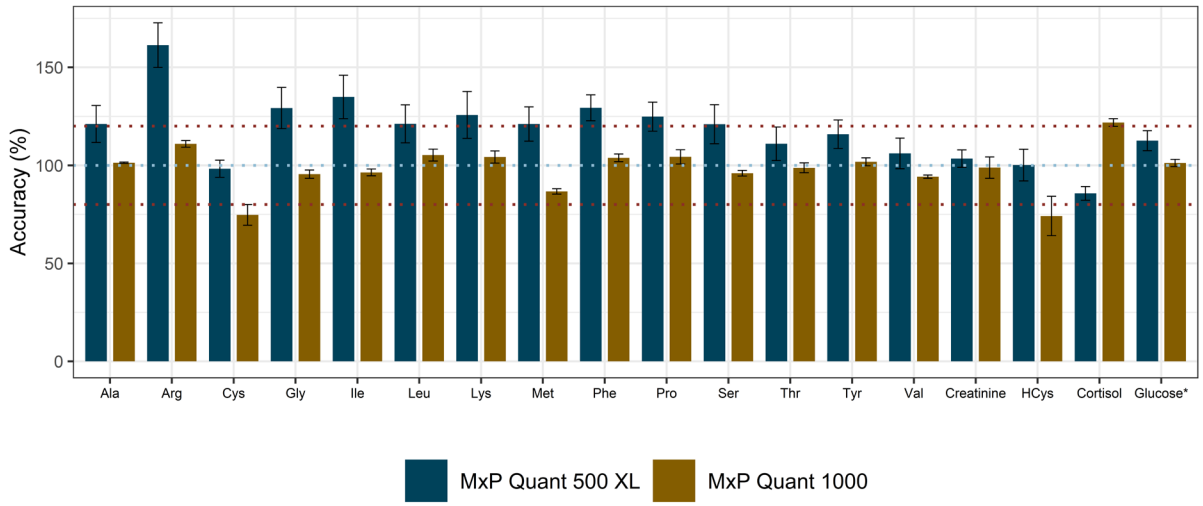

Figure 6: Accuracies of NIST SRM 1950 measured with MxP® Quant 500 XL and MxP® Quant 1000 kits

4 Conclusions

This comparison of the MxP® Quant 500 XL and MxP® Quant 1000 kits demonstrates a high level of continuity across kit generations in terms of detectability, comparability, and reproducibility. Both kits showed strong reproducibility with low coefficients of variation across analytes, confirming the robustness of the standardized biocrates workflow. Most metabolites correlated very well between kits (regression factors >0.9), and detectability was largely consistent across the shared panels.

A limited number of metabolites displayed notable concentration differences, which can be attributed to methodological refinements in the MxP® Quant 1000 kit, including expanded use of calibration standards, the introduction of 3-NPH derivatization for selected metabolite classes, optimized internal standard assignment, and a dedicated lipid plate without derivatization reagents.

These refinements contribute to enhanced quantification accuracy and robustness, even if they result in shifts in absolute concentration values for certain analytes compared to the MxP® Quant 500 XL.

Importantly, while these changes provide clear advantages for the MxP® Quant 1000 kit, the results also confirm that the MxP® Quant 500 XL kit continues to deliver highly reproducible data across small molecules and lipids. Users can therefore have confidence in both datasets, with the MxP® Quant 1000 offering an expanded panel, improved calibration, and enhanced precision for future studies, while maintaining continuity with data generated using the MxP® Quant 500 XL kit.