Automated sample preparation using biocrates' MxP® Quant 500 kit with Waters Andrew+™ Pipetting Robot

Zachary J Mayer, Timothy Griffin, Candace Guerrero;
University of Minnesota, Center for Metabolomics and Proteomics, Minneapolis, USA

Giorgio Horak, Vincent Bel;
Waters Corporation, Vernier, Switzerland

Markus Langsdorf, Stephen Dearth;
biocrates life sciences ag, Innsbruck, Austria

1 Introduction

Standardized protocols and methods capable of generating reproducible results are essential for biological and clinical research in numerous fields, many of which involve large sample cohorts. However, sample preparation remains an obstacle to many applications to large-scale studies. Automated sample preparation using robotics can improve reproducibility and throughput while reducing personnel time for such high-throughput analytical methods. To achieve these benefits and to improve applications to large-scale studies, a protocol for the automated preparation of the MxP® Quant 500 kit has been developed using Waters Andrew+ Pipetting Robot and associated devices.

MxP Quant 500 is a ready-to-use and quality-controlled kit for quantitative metabolic profiling. It targets 630 metabolites across 26 biochemical classes of lipids and small molecules, as well as 264 predefined sums and ratios for advanced biological interpretation. The kit is enhanced with the WebIDQ workflow manager, a companion cloud software that guides users through the entire workflow.

The Andrew+ offers fully automated pipetting, as well as more complex manipulations, using a wide range of dominos and electronic pipettes. It executes OneLab™ Protocols, enabling rapid transition from laborious manual procedures to error-free, robotic workflows. The performance results and gain in efficiency are presented in this application note.

2 Materials and method

Pooled human plasma, human stool, mouse adrenal gland tissue, and macrophage cell pellets were obtained from independent pilot studies. Sample matrices were prepared and measured in replicates (n=6), along with the MxP® Quant 500 kit QCs (n=5). The kit was prepared according to the user manual with 10 µL of sample pipetted per well followed by derivatization, extraction, and finally dilution into two separate measurement plates: one for LC-MS/MS and one for FIA-MS/MS. Each sample was run on four kit plates (three prepared using Andrew+ and one prepared manually), which were each prepped and run on different days.

Automated sample preparation was performed using Waters Andrew+ Pipetting Robot with Extraction+™ and Microplate Shaker+™ associated devices. Andrew+ Protocols were developed and executed using OneLab Software. For details visit MxP® Quant 500 kit automated sample prep method - Protocol - OneLab (andrewalliance.com)

Measurements were performed using an Agilent 6496C LC-QQQ-MS coupled to an Agilent 1290 Infinity II UHPLC system. Data files were directly processed in WebIDQ with

automated quantification, validation, and normalization. Statistical analysis was conducted using R modules. The performance of the method was assessed through multiple metrics to determine the reproducibility of the kit using the Andrew+ Platform.

3 Results and discussion

Detectability with various sample matrices

The Andrew+ is compatible with multiple common sample matrices. The automated protocol produces comparable results to manual preparation and the metabolite detection rates are nearly identical (Figure 1 and Figure 2). Any differences observed between the two sample preparation methods were very minor. For example, when analyzing cells, sample preparation using the Andrew+ Pipetting Robot did yield slightly better detection for amino acids, ceramides, and cholesterol esters.

Reproducibility

All samples measured in replicates showed a median CV below 15% for metabolites measured above LOD (Figure 3). The automated method yielded comparable reproducibility to the manual preparation. The average difference between the manual and automated preparation was 2.8% while CVs differed by 1.1%. Additionally, the automated method provided better accuracy in the external standards samples.

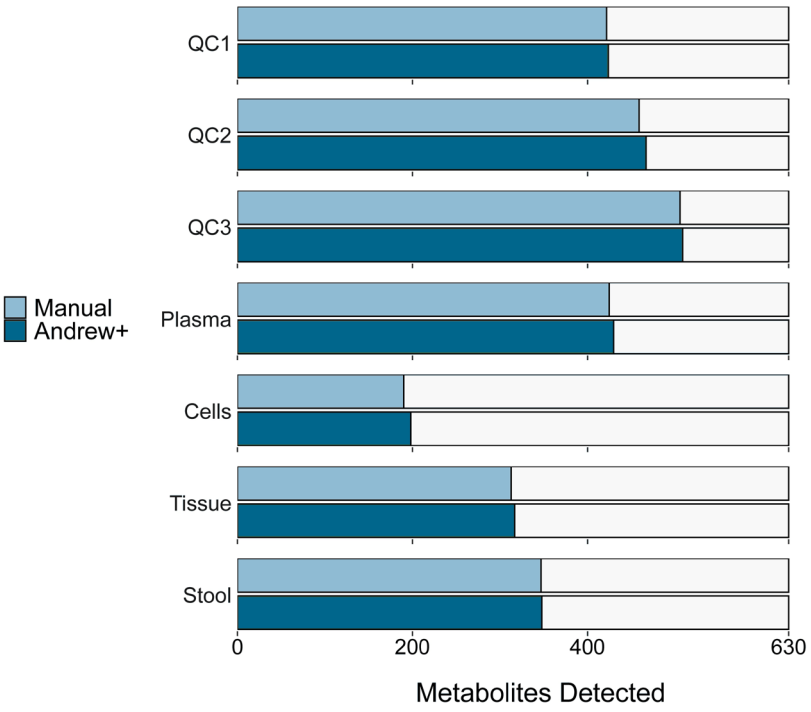

Figure 1: Metabolite detection rates for automated Andrew+ protocol and manual preparation across sample matrices tested

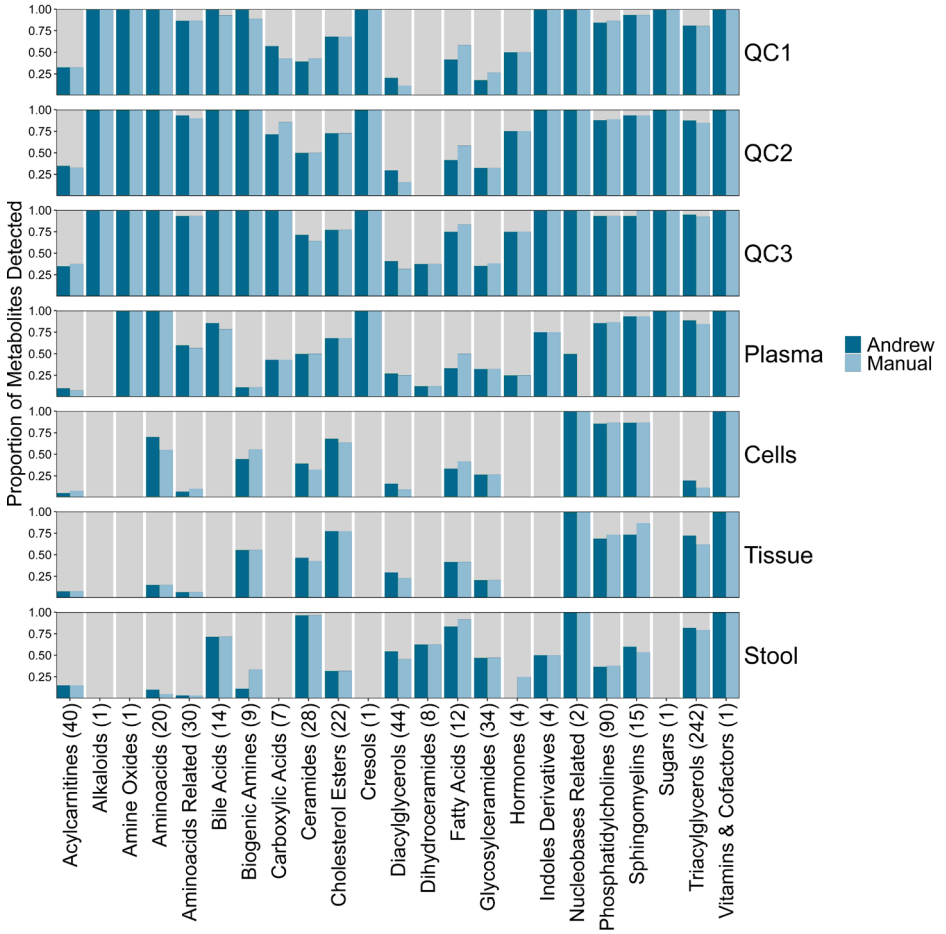

Figure 2: Detection profile of various sample types across different metabolite classes

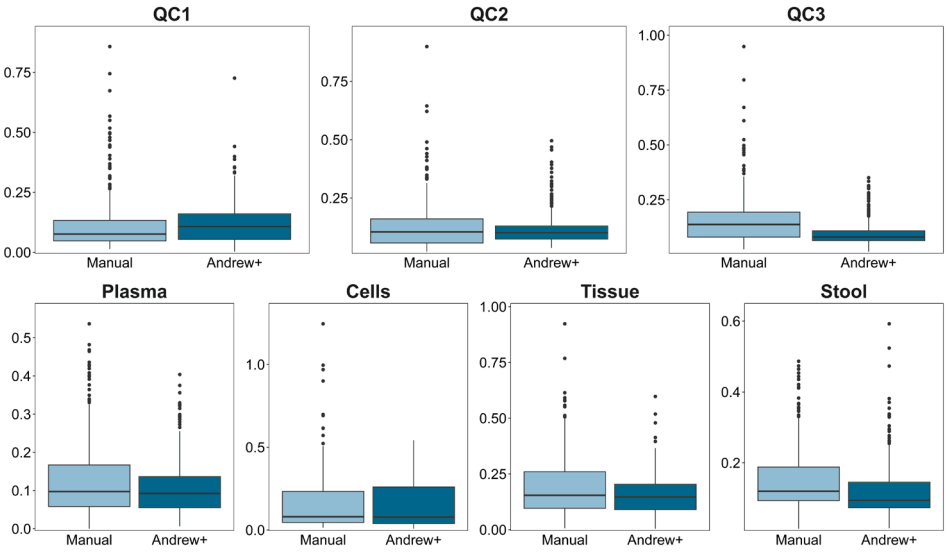

Figure 3: Coefficients of variation (CV) of metabolite concentrations measured for automated Andrew+ protocol and manual sample preparation

Time saving

The automated method's impacts on sample throughput and ease of implementation were also evaluated. The automated method saved time and showed easier implementation compared to the manual preparation. Manual preparation of the kit took ~6 hours and required ~3 hours of hands-on work. The automated preparation took ~5 hours and required ~1 hour of hands-on work (Table 1). This time save makes preparing two kits within one 8-hour period feasible by a single researcher.

	Manual	Andrew+
Time, total	~6 hours	~5 hours
Time, hands-on	~3 hours	~1 hour

Table 1: Preparation time needed for automated Andrew+ protocol and manual sample preparation

4 Conclusions

The automated method yielded comparable results to the manual preparation in terms of metabolite detection and reproducibility across all sample types tested. The automated preparation improved throughput, reduced personnel time and was also easier to perform for inexperienced researchers, whereas manual preparation could take longer for researchers unfamiliar with the preparation.