TECHNICAL NOTE

SAPIENT

Quality analysis across more than **40,000 human biosamples**

The use of mass spectrometry for biomarker discovery is crucial to accelerate drug development, enabling identification of dynamic markers of disease and drug response over time. To date, however, a lack of robust quality metrics across large clinical studies – particularly those involving multiple sites and samples analyzed in independent batches – has precluded the application of mass spectrometry for large-scale biomarker discovery in human studies.

Herein we describe the implementation of a **comprehensive, systematic quality matrix for quality control (QC)** in Sapient's nontargeted mass spectrometry workflows and its application across tens of thousands of human biological samples, demonstrating robust and reproducible data with minimal batch variance across time, instruments, and operators.

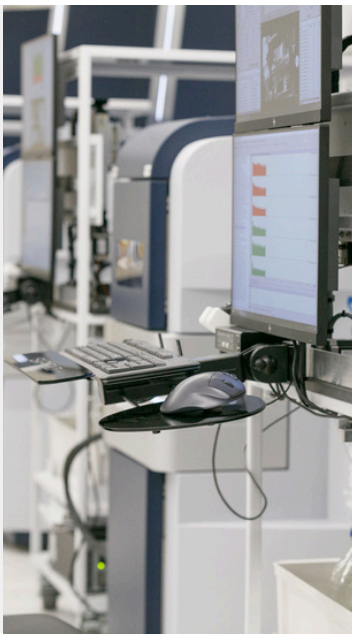
Methodology

Using Sapient's LC-MS workflows, greater than 40,000 diverse human biosamples collected at different time points were analyzed in six independent batches as part of a longitudinal human study. Over 35,000 molecular biomarkers, including metabolites and lipids, were evaluated within the population, making this study **among the largest and deepest nontargeted mass spectrometry analyses of humans to date.**

>40,000
human samples analyzed

6
independent sample batches analyzed at different time points

>35,000
molecules analyzed across samples



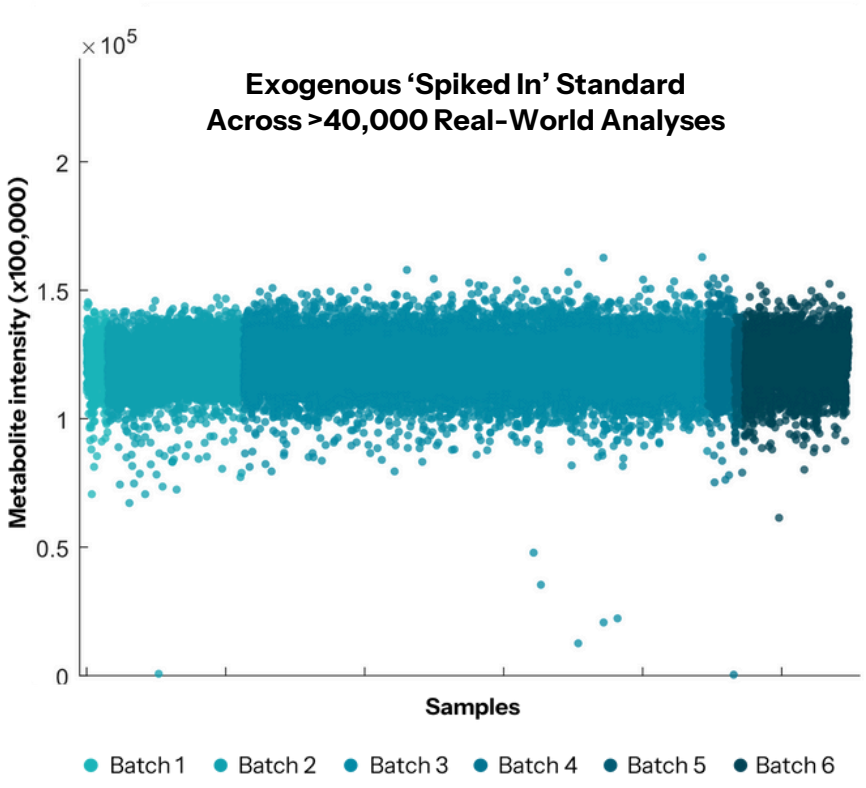
Quality Metrics

Sapient deploys an extensive quality matrix that enables evaluation of sample integrity, sample preparation and handling, automated pipetting, and biomarker extraction, as well as monitoring of chromatographic and mass spectrometry performance at scale and in real time. **More than 500 parameters are continuously monitored,** with quality metrics that include pooled replicate samples analyzed throughout the study as well as exogenous system performance standards that are introduced, or 'spiked in', to each of the 40,000+ biological samples.

Sapient's exogenous standards mix is specifically selected and introduced into biological samples at various points during sample handling to enable close monitoring of our automated workflows and to capture critical quality information at each step. These standards monitor overall system performance as well as sample-to-sample matrix effects, exogenous compounds that may influence mass spectrometry measures, sample oxidation, hemolysis, lipemia and freeze-thaw, and column chromatography.

Systems Performance QC							
	Threshold	**Batch 1**	**Batch 2**	**Batch 3**	**Batch 4**	**Batch 5**	**Batch 6**
Standard 1 – Positive Mode	CV ≤ 20%	7.5%	9.0%	7.5%	6.4%	7.1%	5.4%
Standard 1 – Negative Mode	CV ≤ 20%	9.8%	9.7%	8.8%	11.0%	10.2%	18.9%
Standard 2 – Positive Mode	CV ≤ 20%	5.5%	6.0%	9.6%	12.5%	6.1%	8.7%
Standard 2 – Negative Mode	CV ≤ 20%	3.8%	6.9%	4.5%	8.6%	9.1%	5.1%

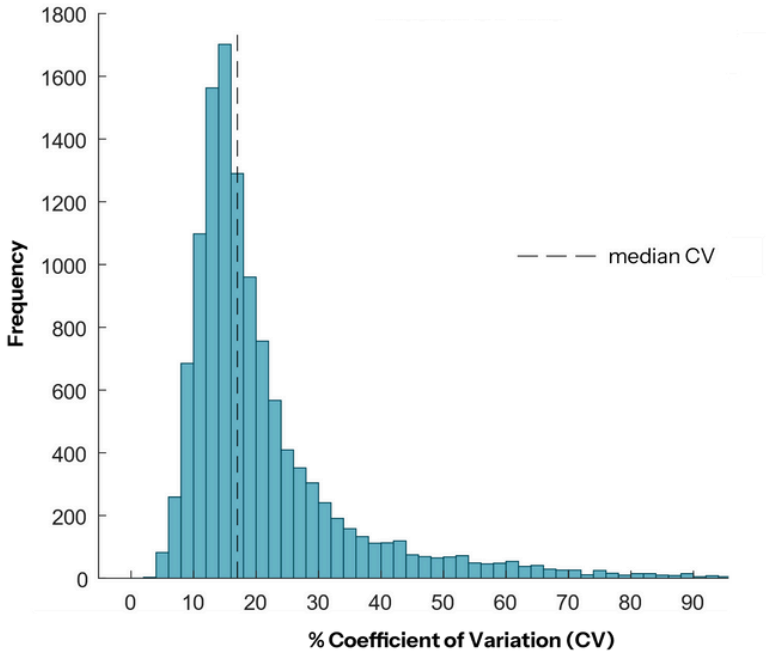
Measure of the exogenous systems performance standards across all 40,000+ biological samples within the 6 batches **demonstrates exceptional robustness, with limited sample-to-sample, day-to-day, instrument-to-instrument, operator-to-operator, and batch-to-batch variance,** for both measures in positive mode and negative mode ESI, achieving less than 20% technical variance across studies.



In addition, evaluation of intensity measures for exogenous, spiked in standard in each of the 40,000+ individual samples (left figure) further supports the technical robustness, **with less than a 0.01% sample failure rate** and limited plate-to-plate and batch-to-batch drift over time.

To assess inter-assay reproducibility, pooled replicate human samples were introduced every ~45 samples, representing over 900 samples in total across the 40,000+ sample study. For more than 12,000 features measured across these blinded replicate samples, Sapient's instrumentation and systems **demonstrate minimal sample handling, chromatographic, or mass spectrometry drift,** with a median coefficient of variation (CV) of 17%. Additionally, 63% of these features demonstrated a technical CV of <20%, confirming the robustness and reproducibility of the analytical measures even across a large human population-based study.

CV Distribution for Mass Spectrometry Measures in Pooled Replicate Samples



Conclusion

Robust QC metrics are critical to ensure accurate data for large-scale biomarker discovery. Sapient has put in place a premier quality matrix that enables multi-parameter evaluation and real-time monitoring of all aspects of our mass spectrometry-based workflow, from sample integrity through to data generation and analysis.

Herein we **demonstrate the effectiveness of our quality matrix across more than 40,000 diverse human biosamples collected longitudinally and analyzed in six independent batches.** The QC results confirm the ability of our LC-MS systems and workflows to maintain exceptional robustness at scale, with minimal instrument or analytical drift, thereby instilling confidence in biomarker findings and insights generated through the study.

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