

INTRODUCTION

Community engagement is needed to address current challenges in quality assurance (QA) and quality control (QC) in untargeted metabolomic studies. Eight laboratories performing untargeted metabolomics, from academic, government and commercial organizations, contributed the details of their QA/QC protocols.

The **ultimate goal** is to create greater visibility into critical QA/QC processes and start the process of establishing good practices.

Untargeted metabolomics: Discovery-based studies that investigate hundreds or thousands of known and unknown metabolites to generate targets for further study.

mQACC Mission Statement

- To engage the metabolomics community to communicate and promote the development, dissemination and harmonization of best quality assurance (QA) and quality control (QC) practices in untargeted metabolomics.

mQACC Objectives

- To establish mechanisms to enable the metabolomics community to adopt QA/QC best practices
- To promote and support systematic training in QA/QC best practices for the metabolomics community.
- To encourage the prioritization and development of reference materials applicable to metabolomics research.

USE OF QC SAMPLES AND STANDARDS

Process blanks: Blanks are routinely prepared solvents and chemicals without biological sample, used to identify system contaminants and batch-to-batch carryover of biological sample. All eight laboratories reported utilizing blanks. At a minimum, blanks were analyzed once at the beginning of the batch, but most (7/8) laboratories embedded additional blanks at the end and/or within the sequence, up to every 5 experimental samples, but more routinely every 10 – 20 samples.

Pooled QC Sample: Pooled QCs are used to condition the platform, for intra-study reproducibility measures, and to correct for systematic errors. All eight laboratories analyzed pooled QCs at the beginning, end and throughout the experimental batch. There was diversity in the frequency and total number of pooled QC samples analyzed per batch (Table 1). Some also used this pooled sample to condition the columns prior to analysis and to “warm-up” the column prior to batch analysis.

Table 1. Frequency of Pooled QC use per batch

Percentage of laboratories	QC Pool Frequency
50%	Multiple times throughout batch
25%	Every 5-10 experimental samples
13%	Every 10 experimental samples
13%	Every 20 experimental samples

Long Term Reference (LTR) Material: LTR is used for inter-laboratory and inter-study data assessment. Three laboratories reported using a LTR sample (e.g. NIST SRM 1950) to assess and monitor long term instrument stability and performance. These were run at lower frequency than other QC samples, but were still included in every batch.

Internal Standards: Internal standards are used to assess system stability per sample. Most (7/8) laboratories reported using internal standards (isotopically labeled endogenous compounds or non-endogenous compounds) spiked into all samples analyzed (range per method 2-12).

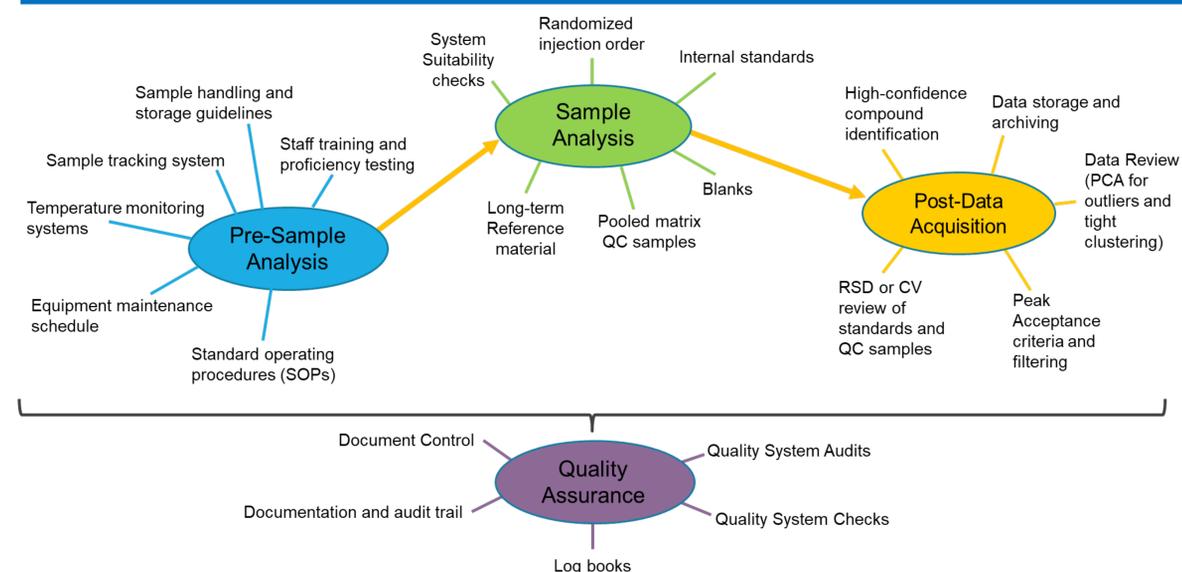
Experimental Sample Replicates: One laboratory reported that they analyzed select experimental samples in duplicate or triplicate to further assess method reproducibility and stability.

DATA ACCEPTANCE CRITERIA

Peak Acceptance: Half of the laboratories reported filtering experimental peak data using the QC pooled samples. An example criteria for peak acceptance for further analysis was experimental peak detection in $\geq 70\%$ of the QC pooled samples and/or having had an $RSD \leq 15, 20$ or 30% (respective of different laboratories) in the replicates. Three laboratories reported requiring experimental peaks exceeding blank levels by $>3 \times$ response. Two laboratories highlighted the importance of manual review of peaks and alignment for peak inclusion.

Principle Component Analysis (PCA): Several laboratories reported using PCA to assess dataset quality: visualizing the controls, blanks, replicates, etc., for tight clustering and to inspect for outliers.

SUMMARY OF mQACC QA/QC PROCEDURES



COMPOUND IDENTIFICATION

Compound Identification: All laboratories noted the importance of confirming metabolite identification using authentic standards. Half of the laboratories reported creating an in-house authentic standard database where retention time, mass and fragmentation spectra were documented. Experimental data was then searched against the in-house libraries for Metabolomics Standards Initiative (MSI) level 1¹ confidence identifications. Individual in-house compound database sizes varied between 500 to 4000 compounds. Two laboratories reported searching experimental peak data against publicly available databases (eg Metlin and Lipid Maps), but noted that these were then confirmed by comparison to an authentic standard. This highlights the fact that mass (m/z) only identifications are considered lower confidence and have a higher probability of being a false positive.

OTHER QUALITY SYSTEM PROCESSES

- All laboratories reported using standard operating procedure (SOP) documentation
- Several laboratories highlighted documentation throughout the entire workflow from sample preparation to informatics data manipulation for traceability
- Three laboratories reported having repeated formal training and proficiency testing to qualify and certify that researchers were able to perform required tasks
- Three laboratories reported having a formal review and QA sign-off process to ensure appropriate procedures were followed and to check for errors
- Three laboratories reported establishing LIMS type systems for tracking, storing and archiving data from sample receipt to final report for full traceability
- Two laboratories reported establishing and documenting a maintenance, calibration and tuning schedule for all equipment including pipets, balances and instrumentation
- Three laboratories reported having temperature monitoring and alarm systems in place for freezers, refrigerators and laboratories

CONCLUSION

- QA and QC practices are fundamental to ensuring high quality, reproducible data from untargeted metabolomics. The QA/QC procedures, even within this small group of contributors, varied significantly. This highlights the importance of identifying, cataloging, harmonizing and disseminating QA/QC best practices with full involvement of the community.
- Leading laboratories have established QA and QC systems that span pre-sample and sample analysis and post-data acquisition, with underlying QA documentation and system checks. Encouragingly, many of their quality systems have commonalities, such as widespread use of internal standards and pooled QC samples, and speaks to the benefits of these procedures when utilized in untargeted metabolomics.