Metabolomics (2023) 19:93
https://doi.org/10.1007/s11306-023-02060-4

Metabolomics 2022 workshop report: state of QA/QC best practices in LC–MS-based untargeted metabolomics, informed through mQACC community engagement initiatives

Warwick B. Dunn[1] · Julia Kuligowski[2] · Matthew Lewis[3] · Jonathan D. Mosley[4] · Tracey Schock[5] · Candice Ulmer Holland[6] · Krista A. Zanetti[7] · Dajana Vuckovic[8] on behalf of Metabolomics Quality Assurance and Quality Control Consortium (mQACC)

Received: 22 May 2023 / Accepted: 18 October 2023

Abstract

Introduction The Metabolomics Quality Assurance and Quality Control Consortium (mQACC) organized a workshop during the Metabolomics 2022 conference.

Objectives The goal of the workshop was to disseminate recent findings from mQACC community-engagement efforts and to solicit feedback about a living guidance document of QA/QC best practices for untargeted LC–MS metabolomics.

Methods Four QC-related topics were presented.

Results During the discussion, participants expressed the need for detailed guidance on a broad range of QA/QC-related topics accompanied by use-cases.

Conclusions Ongoing efforts will continue to identify, catalog, harmonize, and disseminate QA/QC best practices, including outreach activities, to establish and continually update QA/QC guidelines.

Keywords Metabolomics · Quality control (QC) samples · Liquid chromatography–mass spectrometry (LC–MS) · Internal standards · Blanks · System suitability testing (SST) · Analytical batch

1 Introduction

The development and dissemination of quality assurance (QA) and quality control (QC) practices in targeted and quantitative small molecule analysis have been successfully implemented and refined during the last twenty years and are currently at a mature status (for example, European Medicines Agency, 2018; US Food and Drug Administration (FDA) 2018). The development of QA and QC best practices for untargeted small molecule analysis is ongoing in untargeted metabolomics applications. While earnestly in development (Broadhurst et al., 2018), these QA and QC best practices have not reached general agreement globally nor a mature status. There is a critical need to

✉ Warwick B. Dunn
warwick.dunn@liverpool.ac.uk

[1] Department of Biochemistry, Cells and Systems Biology, Centre for Metabolomics Research, Institute of Systems, Molecular and Integrative Biology, University of Liverpool, Biosciences Building, Crown Street, Liverpool L69 7ZB, UK

[2] Neonatal Research Group, Health Research Institute La Fe, Avda. Fernando Abril Martorell 106, 46026 Valencia, Spain

[3] Bruker Life Sciences Mass Spectrometry, Bruker UK Ltd, Welland House, Longwood Close, Coventry CV4 8AE, UK

[4] Center for Environmental Measurement and Modeling, Environmental Protection Agency, Athens, GA, USA

[5] Chemical Sciences Division, National Institute of Standards and Technology (NIST), Charleston, SC 29412, USA

[6] Eastern Laboratory, Office of Public Health Science (OPHS), Food Safety and Inspection Service (FSIS),, U.S. Department of Agriculture (USDA), Athens, GA 30605, USA

[7] Office of Nutrition Research, Division of Program Coordination, Planning, and Strategic Initiatives, Office of the Director, National Institutes of Health, Bethesda, MD, USA

[8] Department of Chemistry and Biochemistry, Concordia University, 7141 Sherbrooke Street West, Montreal, QC H4B 1R6, Canada

Published online: 08 November 2023

standardize, disseminate, implement, and provide training in QA and QC best practices in untargeted metabolomics studies to ensure high quality data generation, analysis, and reporting. To assist in these goals, the Metabolomics Quality Assurance and Quality Control Consortium (mQACC) was formed in 2018 following a workshop held at the National Institutes for Health in 2017 (Beger et al., 2019).

mQACC is an international consortium with more than 90 members, driven by a mission to engage the metabolomics community to communicate and promote the development, dissemination, and harmonization of QA/QC best practices in untargeted metabolomics (https://www.mqacc.org/). mQACC operates through distinct working groups (WGs). The Best Practices WG was established to identify, catalogue, harmonize, and disseminate QA/QC best practices for untargeted metabolomics, which are agreed upon in the metabolomics community (Fig. 1).

The Best Practices WG has been and continues to engage with the metabolomics community in multiple complementary ways including questionnaires, online and face-to-face workshops and interactive forums and through face-to-face workshops at national and international conferences to discuss QA and QC in metabolomics. One recent face-to-face workshop was held at Metabolomics 2022, the 18th annual conference of the Metabolomics Society, in Valencia, Spain on June 19th 2022. The objectives of the workshop were to 1) disseminate findings from the mQACC Best Practices Working Group's extensive community engagement efforts in relation to LC–MS untargeted metabolomics; and 2) solicit further feedback from the international metabolomics community on the compiled and summarized findings to establish a best practices living guidance document that will be freely accessible to researchers.

The objective of this workshop report is to further disseminate information obtained throughout this community engagement process and further engage scientists in the field on QA/QC best practices in metabolomics.

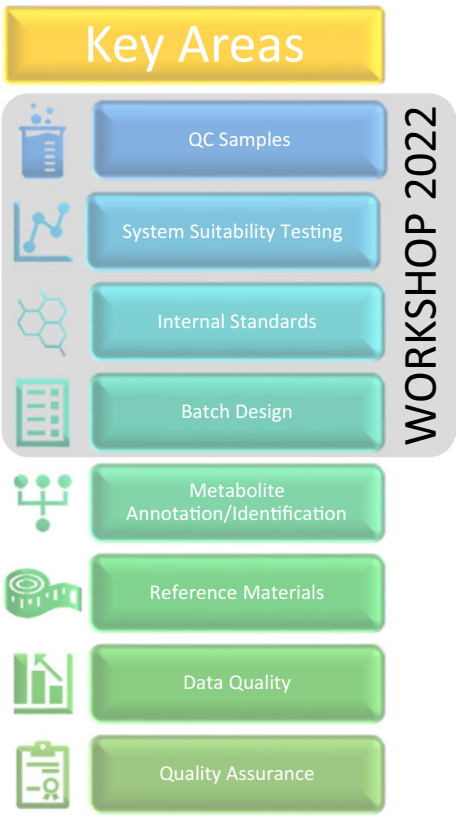

Fig. 1 Metabolomics QA/QC key areas

2 Workshop structure

The workshop was structured to report on the information gathered on four key QC topics through the mQACC Best Practices WG community engagement activities and gather additional insight from workshop attendees on each topic. To accomplish these goals, it was delivered by six mQACC members across a two-hour period and designed to be interactive between the workshop organisers and attendees. With over 200 scientists in attendance, mQACC was introduced to the audience during a 5-min presentation. This was followed by four 25-min sessions, each structured to include an introduction to a specific topic, presentation of key findings from community engagement efforts, polling questions for the audience managed through the EventsAIR mobile phone application, and a facilitated 10-min discussion that included Q&A. The four topics presented were (1) pooled and intra-study QC samples; (2) system suitability evaluation; (3) use of internal standards and (4) design of the analytical batch, as highlighted in Fig. 1. Over 140 attendees responded to each poll question. The polls were recorded, and the results are available in Fig. 2. There was high engagement by the workshop attendees as evidenced by the wide range of comments and feedback received during the discussion.

3 Workshop content

In this workshop, four distinct QA/QC key areas were addressed as outlined below. The presentations provided for each of the four topics are available in Supplementary File 1 and the responses to the polls are available in Fig. 2.

In the workshop, the first presentation focused on pooled and intra-study QC samples. Pooled and intra-study QC samples are typically created by combining small aliquots of each biological test sample or a representative subset of all study samples to create a single QC sample, which is then processed in the same way as the biological test samples (Broadhurst et al., 2018; Viant et al., 2019).

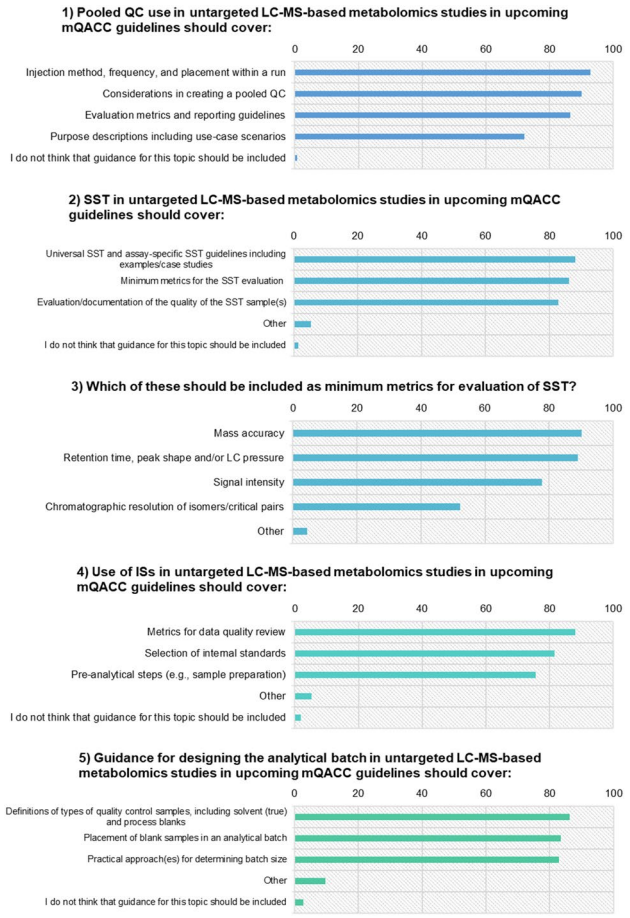

Fig. 2 Polling questions administered to the audience during the workshop by using an on-line tool. Note: All questions were 'choose all that apply' questions. Number of responses N = 140 (Question 1); N = 151 (Question 2); N = 153 (Question 3); N = 152 (Question 4); and N = 146 (Question 5) participants

Data from the analysis of pooled QC samples can be used for batch correction, filtering, system conditioning, and metabolite annotation, among others. The creation of the pooled QC sample is dependent on the type of sample matrix, and the step at which it is generated affects how the data can be used (i.e., correction of either instrument variance or extraction and instrument variance). The number of QCs injected at the beginning and during an analytical batch, and the injection method and placement of pooled QCs within the sequence are specific to the instrument and method used. Precision of data from pooled QC samples is currently only assessed visually using principal component analysis, but future developments to include quantitative metrics for data evaluation and reporting may be considered. Dilution series of pooled QC are currently not used broadly but provide objective evidence of signal linearity (Croixmarie et al., 2009; Lewis et al., 2016).

The second presentation focused on system suitability testing (SST), which includes all activities performed prior to analyzing study samples to ensure that the analytical system is fit for purpose and within specification. SST is used to assess the operation and lack of contamination of the analytical platform (Broadhurst et al., 2018; Viant et al., 2019), helping to minimize the loss of precious samples if the instrument/assay is not performing up to specification. Universal SST ensures that the instrument is operating within certain specifications, while assay-specific SST assesses instrument suitability for a particular project. The majority of the metabolomics community currently performs both types of SST. SST needs to be passed before study samples are analyzed, and appropriate corrective measures need to be taken if it fails (Evans et al., 2020). Both in-house and commercial standard mixtures and biological samples can be used for SST, and evaluation metrics should include retention time, mass, and sensitivity assessments using predefined acceptance criteria. There is currently no community-wide agreement on metrics to use or acceptance criteria, and additional commercial mixtures and better longitudinal monitoring software/record-keeping are needed.

The third presentation discussed the use of internal standards (IS) as a widely adopted QC measure in the metabolomics community, with increased importance in large multi-batch studies. Different types of IS are used, including isotopically labeled compounds, exogenous compounds, and isotopically labeled extracts, with the selection and number of ISs used being method dependent. Similar to pooled QC samples, the step at which ISs are added in the workflow determines the use of derived data (i.e., evaluation of either instrumental variance or extraction and instrumental variance) and ISs allow for assessing data quality through various metrics, such as peak shape, retention time drift, signal response, mass accuracy, stability, and matrix effect. The IS signal can be compared to pre-established cutoff criteria for outlier elimination and batch acceptance. However, there is still no community consensus on how to use IS during post-processing. While there is need to build consensus on common ISs to improve intra- and inter-lab comparisons and facilitate data sharing, community interactions have shown there may be a lower barrier to establishing a common set of metrics for assessing data quality, regardless of the selection and number of ISs.

The final presentation focused on designing an analytical batch. We defined analytical batch as a series of continuously analyzed samples. An analytical batch typically includes various types of samples, such as blank, pooled QC, and study samples, as well as technical replicates injected according to specific batch design. Blank samples, i.e., solvent/true blanks or process blanks, are used to evaluate background contaminants, carryover, and cross-contamination between samples. Blank samples should be included at the

beginning and end of a batch, though a recent study suggests they should not be used in-between samples to avoid disturbing column conditioning (Martínez-Sena et al., 2019). The batch size depends on various factors, such as the matrix type, instrument stability, and staff schedule, and needs to be determined during assay development by monitoring signal loss and establishing a cutoff criterion.

4 Attendee engagement

The audience participated in discussions after each topic was presented, providing feedback, ideas, personal experiences, and constructive criticism of the data presented. Some relevant aspects not covered in the presentations included the importance of checking mass calibration (particularly in ESI- mode), the significance of SST for shared facilities where proteomics and metabolomics studies are applied on the same instrument, the assessment of carryover, and using zero blanks in addition to solvent blanks. Practitioners highlighted issues related to retention time shifts and suggested using the retention time and signal intensity of features to determine the number of QCs needed for system conditioning. SST was also discussed with respect to the acquisition of MS/MS data to implement QCs for metabolite annotation early in the experimental workflow. The feedback received pointed out several issues including the need for alternative approaches to the repeated injection of pooled QC samples as this practice substantially enhances the number of injections; the limitations of IS usage related to availability and cost of isotopically labelled ISs; and challenges related to the use of pooled QC samples for feature filtering when targeting low-concentration analytes, particularly in the field of exposomics. The workshop polls clearly demonstrated that the metabolomics community would like to see a broad coverage of information in upcoming mQACC guidelines as demonstrated by more than 70% of responders requesting all provided options in all five poll questions (see Fig. 2). There was one exception in the SST section (Question 3d) where chromatographic resolution of isomeric pairs reporting was only requested by 50% of responders.

5 Future actions

Based on the reported polls, mQACC guidance documents will include information on (1) definitions, (2) preparation of QC samples and ISs, (3) analysis of QC and blank samples in batches, (4) data interpretation and acceptance criteria for QCs, SST, and ISs, and (5) reporting QC-related data. It should be noted that mQACC has a task group dedicated to harmonizing and disseminating QA/QC reporting guidelines, who published reporting guidelines for QC samples in 2022 (Kirwan et al., 2022). Furthermore, a clear need for inclusion of use-cases has been expressed by the participants.

During the final discussion, the need for detailed guidelines, similar to those available for targeted bioanalytical assays, was emphasized to consolidate the use of QC samples in untargeted LC–MS-based metabolomics. While the community recognizes the importance of QA/QC in this area, concerns were raised regarding minimum requirements, as well as the associated effort in terms of time and cost. Due to the diversity of applications, methods, and instruments employed, and with the aim of being inclusive, the mQACC guidelines seek to provide useful tools to practitioners rather than being prescriptive. Therefore, the focus of the living guidance will be on training scientists and improving the way they perform and report their experiments rather than establishing strict minimum requirements. Ongoing efforts to identify, catalog, harmonize, and disseminate the QA/QC best practices, including outreach activities to scientists working in this field, will continue in the future to establish and update QA/QC guidelines. These efforts included a mQACC organized workshop during the Metabolomics 2023 conference which focused on the following topics; (1) metabolite annotation/identification, (2) reference materials, (3) data quality review and (4) quality assurance (Fig. 1).

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11306-023-02060-4.

Acknowledgements The authors would like to thank the Metabolomics Society and the Metabolomics 2022 scientific organising committee for the opportunity to host the workshop.

Author contributions All authors contributed equally to the organization of the workshop. The manuscript draft was prepared by JK and WBD and all authors commented and edited the manuscript and approved submission.

Funding The work presented did not receive any funding. JK acknowledges financial support received from Carlos III Health Institute (Spain) (grant number CPII21/00003). DV acknowledges financial support from Fonds de Recherche du Quebec—Sante (FRQS) (Chercheur Boursier Junior 2 grant 312947).

Data availability All data are included in the manuscript.

Code availability Not applicable.

Declarations

Competing interests The authors declare no competing interests.

Disclaimer This article reflects the views of the author(s) and does not necessarily represent the views or policies of the U.S. Environmental Protection Agency (EPA) nor the National Institutes of Health (NIH). Certain commercial equipment, instruments, trade names, products or materials are identified in this paper in order to specify the experimental procedure adequately. Such identification is not intended to imply

or convey and should not be interpreted as conveying official approval, recommendation or endorsement by the National Institute of Standards and Technology, the EPA, or the NIH, nor is it intended to imply that the materials or equipment identified are necessarily the best available for the purpose.

References

Beger, R. D., Dunn, W. B., Bandukwala, A., Bethan, B., Broadhurst, D., Clish, C. B., et al. (2019). Towards quality assurance and quality control in untargeted metabolomics studies. *Metabolomics: Official Journal of the Metabolomic Society, 15*(1), 4. https://doi.org/10.1007/s11306-018-1460-7

Broadhurst, D., Goodacre, R., Reinke, S. N., Kuligowski, J., Wilson, I. D., Lewis, M. R., & Dunn, W. B. (2018). Guidelines and considerations for the use of system suitability and quality control samples in mass spectrometry assays applied in untargeted clinical metabolomic studies. *Metabolomics: Official Journal of the Metabolomic Society, 14*(6), 72. https://doi.org/10.1007/s11306-018-1367-3

Croixmarie, V., Umbdenstock, T., Cloarec, O., Moreau, A., Pascussi, J.-M., BoursierNeyret, C., & Walther, B. (2009). Integrated comparison of drug-related and drug-induced ultra performance liquid chromatography/mass spectrometry metabonomic profiles using human hepatocyte cultures. *Analytical Chemistry, 81*, 6061–6069. https://doi.org/10.1021/ac900333e

European Medicines Agency. (2018). Bioanalytical method validation - Scientific guideline. *European Medicines Agency*. Retrieve from 13 February 2023 https://www.ema.europa.eu/en/bioanalytical-method-validation-scientific-guideline

Evans, A. M., O'Donovan, C., Playdon, M., Beecher, C., Beger, R. D., Bowden, J. A., et al. (2020). Dissemination and analysis of the quality assurance (QA) and quality control (QC) practices of LC-MS based untargeted metabolomics practitioners. *Metabolomics: Official Journal of the Metabolomic Society, 16*(10), 113. https://doi.org/10.1007/s11306-020-01728-5

Food and Drug Administration (2018). Bioanalytical Method Validation, Guidance for Industry US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), and Center for Veterinary Medicine (CVM), Retrieve from 13 February 2023 https://www.fda.gov/media/70858/download

Kirwan, J. A., Gika, H., Beger, R. D., Bearden, D., Dunn, W. B., Goodacre, R., et al. (2022). Quality assurance and quality control reporting in untargeted metabolic phenotyping: mQACC recommendations for analytical quality management. *Metabolomics: Official Journal of the Metabolomic Society, 18*(9), 70. https://doi.org/10.1007/s11306-022-01926-3

Lewis, M., Pearce, J. T. M., Spagou, K., Green, M., Dona, A. C., Yuen, A. H. Y., et al. (2016). Development and application of ultra-performance liquid chromatography-TOF MS for precision large scale urinary metabolic phenotyping. *Analytical Chemistry, 88*(18), 9004–9013. https://doi.org/10.1021/acs.analchem.6b01481

Martínez-Sena, T., Luongo, G., Sanjuan-Herráez, D., Castell, J. V., Vento, M., Quintás, G., & Kuligowski, J. (2019). Monitoring of system conditioning after blank injections in untargeted UPLC-MS metabolomics analysis. *Scientific Reports, 9*(1), 1–9. https://doi.org/10.1038/s41598-019-46371-w

Viant, M. R., Ebbels, T. M. D., Beger, R. D., Ekman, D. R., Epps, D. J. T., Kamp, H., et al. (2019). Use cases, best practice and reporting standards for metabolomics in regulatory toxicology. *Nature Communications, 10*(1), 3041. https://doi.org/10.1038/s41467-019-10900-y