Introducing the Lipidomics Minimal Reporting Checklist

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Standfirst:

The rapid increase in lipidomic data has triggered a community-based movement to develop guidelines and minimum requirements for generating, reporting, and publishing lipidomic data. The creation of a dynamic checklist summarizing key details of lipidomic analyses using a common language has the potential to harmonize the field by improving both traceability and reproducibility.

Problems in lipidomics reporting

Since the early 2000s, lipid analysis by mass spectrometry (MS) has undergone substantial growth. This growth has benefited many scientific fields, and now lipid measurements are increasingly common in a diverse array of scientific fields and journals. This expansion has led to confusion and uncertainty regarding published data when analyses are performed. Methods sections are frequently minimized, and methods, when reported, are relegated to supplementary material where they are often truncated and not carefully reviewed. Submissions to non-lipid-focused journals do not always receive technical input from peer reviewers with lipidomic expertise to properly assess the quality of the methods. Furthermore, the expanded use of commercial services that provide lipidomic measurements leads to data being published with little or no description of the methods due to claims of proprietary methodology. A further uncertainty appears when researchers annotate MS data using databases and search algorithms without sufficient knowledge of how the curation is applied or considering the level of accuracy that is ascribed. Not all of these databases or algorithms have been thoroughly vetted by the community and have often been developed for a different purpose than lipid annotation. With few exceptions, lipids are measured with commercially available instrumentation (MS and liquid chromatography) using variations on several over-arching methods. These methods must be described sufficiently for a reader to judge the quality and validity of reported lipidomic data. When researchers who lack lipid expertise receive data from colleagues, databases, or proprietary commercial services, it is often difficult to judge its quality and completeness. This difficulty can lead to the publication of papers with inadequate or incorrect lipid data [1,2].

A move towards standardization

In a previous Commentary we called for the lipidomics community to work together towards standardization in the field by establishing guidelines and minimum requirements for the publication of lipidomics data [3]**.** Established in 2019, the Lipidomics Standard Initiative (LSI) began coordinating these efforts through a series of public web-based workshops over the summer of 2020, which attracted approximately 150 international researchers from both the lipidomic and metabolomic fields. LSI integrated feedback from these meetings to prepare guidelines and minimum reporting standards. These guidelines are also published on the LSI website that is affiliated as interest group to The International Lipidomics Society (ILS, https://lipidomicssociety.org/). Although consensus-driven guidelines for lipidomics are now in place, actionable use of these guidelines remains absent. Here, we propose a checklist concept that leverages and expands these guidelines into a freely available, virtual document to accelerate standardization in our field.

The Checklist Concept

We have compiled the guidelines and minimum requirements into a dynamic, interactive, virtual checklist accessible to everyone (https://lipidomicstandards.org/). The checklist is composed of nine sections.

The **Pre-Analytics** section covers aspects of the samples prior to processing, extraction, or analysis. This section includes sample type, origin, storage conditions, freeze-thaw cycles, and other information pertinent to the quality and integrity of the samples. While researchers cannot always control every aspect of pre-analytics (e.g., biobank samples), this section allows interested parties to rapidly assess issues pertaining to the history of the samples prior to analysis.

Overall Study Design gives a snapshot of the general workflow and types of analytical methods used. Mode of sample introduction (e.g., direct infusion, chromatography) and ionization method (e.g., electrospray ionization, matrix-assisted laser desorption/ionization) and whether the analysis is qualitative or quantitative are covered in this section.

Lipid Extraction includes all aspects of extracting and isolating lipids from biological samples. The extraction method, solvents used, internal standards, and details on additional processing are covered here. This section is particularly important because suboptimal extraction of lipids has a profound negative impact on all downstream analysis steps.

Analytical Platform provides details on the type of MS approach used and details on liquid chromatography. Instrument type and vendor, sample introduction, and any orthogonal dimensions of analysis are described here. Key parameters including instrument resolution, mass accuracy, and acquisition mode will also be included.

Lipid Identification defines how this instrumentation was applied to identify a lipid molecule from chromatographic and/or mass spectral data. MS level (MS^1, MS^2), ionization polarity, isotope correction, retention time, and use of authentic standards are included here. This section of the checklist is unique in that it links to an expanded table where details on the respective lipid classes can be reported. Data such as precursor, fragment ions, data manipulation steps like smoothing, check of background ions and signal to noise level are reported. Like the section on Lipid Extraction, this section is also crucial due to the highly complex and often isomeric and isobaric nature of lipids.

Lipid Quantitation designates how mass spectral data was transformed into quantitative values. Use of calibration curves or relative response, number of standards per lipid class, and use of internal standards are among the checklist items covered.

Finally, **Quality Control**, **Method Validation**, and **Reporting** cover aspects of data and method quality and how the data were reported. The use of blank and quality control samples are included here as well as depth of method validation including dynamic ranges, limits of detection/quantitation. Availability of (raw) is also reported.

An example of the checklist is shown in **Figure 1.** A glossary of terms for each entry accompanies the checklist.

Intent and Implementation

The Lipidomics Minimal Reporting checklist has broad implications and can serve as a pillar for the field, setting standards for ongoing and future work. The checklist has multiple

uses, but we first and foremost recommend that it should be included as supplementary material in publications containing lipidomic data. Modern MS-based lipidomic analysis comes in many flavors and due to the continued growth of the field, few scientists have sufficient training and expertise to fully evaluate manuscripts with lipidomic data. This skill gap poses an essential problem for journal editors and reviewers, mainly when manuscripts with lipidomic data are submitted to multidisciplinary journals or journals focusing on biomedical research. The proposed checklist will give editors and reviewers feedback regarding the quality and completeness of lipidomic data and will, in a planned future update, introduce a numeric and/or color-coded scoring system. Deficiencies in critical areas are flagged so that editors and reviewers know that further guidance from experts in lipidomics might be warranted. We expect this checklist system to improve the review process for journals at no additional cost and minimal effort on their part, as the LSI is committed to maintaining the checklist as a freely available resource.

The checklist also has orthogonal benefits. It serves as an excellent resource for the design of experiments as it encompasses best practices in lipidomics. An experiment designed with the help of the Lipidomics Minimal Reporting Checklist should be of high quality and yield data that are both consistent and interpretable. The checklist can also be used to prepare a manuscript to ensure that lipidomic data reported are at the highest level of rigor. Finally, the checklist represents a valuable educational tool for both the inexperienced and expert lipid mass spectrometrist, serving as a continually updated, central repository of best practices that can be used as the foundation for the education of students, fellows, and colleagues.

We developed the checklist to be informative yet require only a comparatively short amount of time to complete. The Lipidomics Minimal Reporting Checklist is not meant to recapitulate or replace the methods section in a manuscript but instead offers an easily understandable summary as a guide for readers. The checklist can be completed in 30-60 minutes, depending on the complexity of the experiments and data. If researchers use the checklist to design the experiments and organize their manuscripts, then completion of the checklist will take even less time.

As a virtual resource, the checklist is flexible and will be revised based on feedback from users, evolving in parallel with the lipidomics field. As recently emphasized [2], a myriad of methods exists to analyze lipids, each with its own purpose and merits. We do not intend to dictate how to measure lipids. As a diverse group of scientists with extensive knowledge and expertise in all aspects of lipidomics, we define the minimum requirements necessary to measure lipids and report lipidomic data. This checklist thus constitutes a consensus-driven tool for researchers to navigate this evolving scientific field successfully. By adopting the Lipidomics Minimal Reporting Checklist, the lipidomics field will converge into a stronger, more robust, and harmonious area.

Author contributions

J.G.M., C.S.E., D.K. and M.K. contributed equally, wrote the manuscript, and developed the online checklist. D.K. programmed the online checklist system. J.A., Makoto Arita, Masanori Arita, E.S.B., J.BM., J.A.B., B.B., S.R.E., M.F., W.J.G., X.H., J.H., N.H., J.P.K., H.C.K., T.W.M., V.B.O., Daisuke Saigusa, Dominik Schwudke, A.S., C.Z.U., M.R.W., M.W., D.W. and Y.X. discussed and contributed to the manuscript. R.A., G.L. and K.E. jointly coordinated this work, wrote the manuscript, and developed the online checklist. All authors annotated data and approved of the final manuscript.

Conflict of interest

Kim Ekroos is the owner of Lipidomics Consulting Ltd. The authors declare no conflict of interest.

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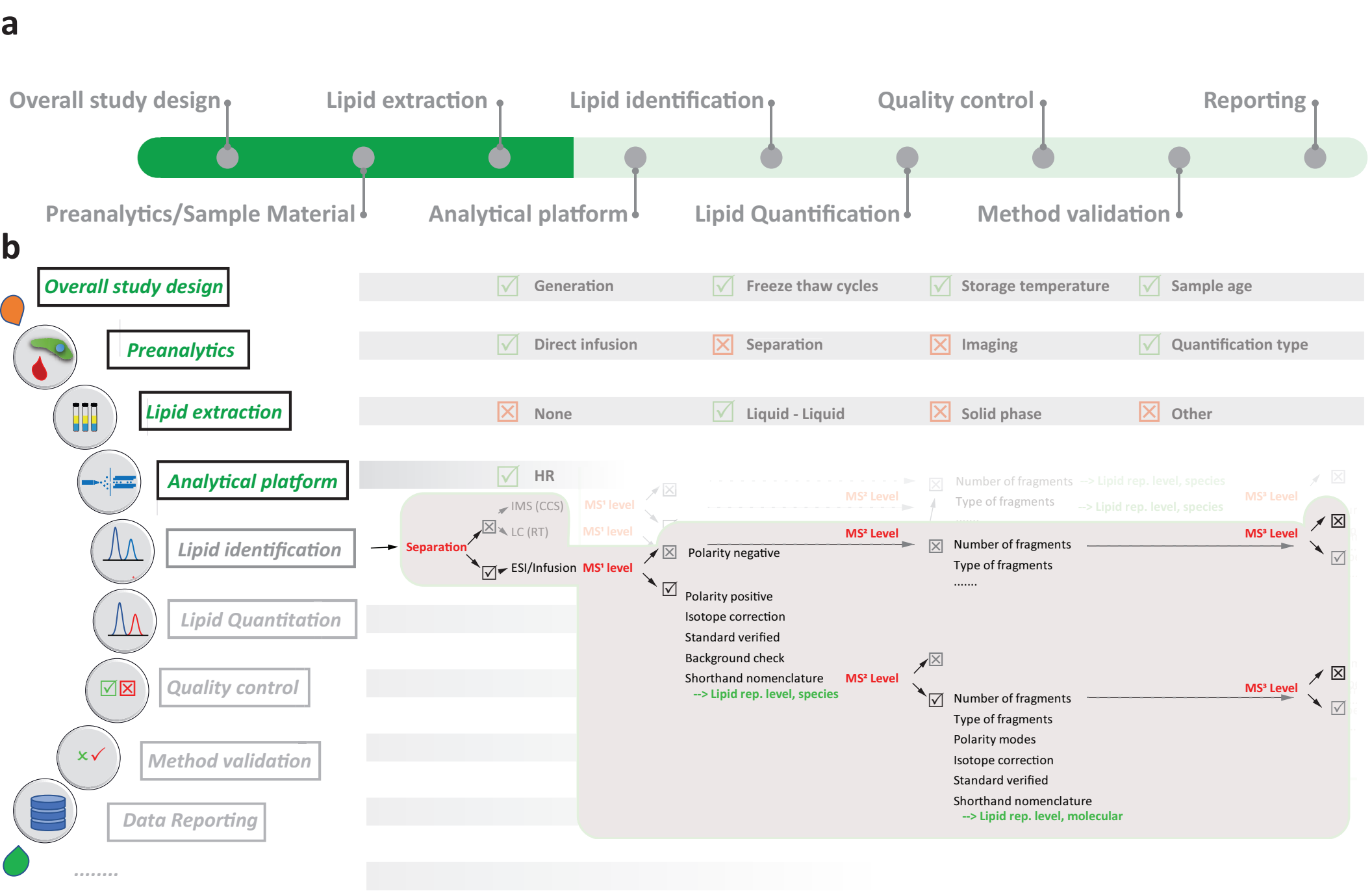
Figure 1. Graphical overview of the Lipidomics Minimal Reporting Checklist concept. (a) A progress bar illustrates the various sections of the lipidomics checklist. (b) The nine major categories that are necessary to conduct lipidomic research are shown on the left side. An expansion of the “Separation” checkbox within the “Lipid Identification” section shows a decision tree with subcategories and dropdown menu options for this section that are available on the online platform. After completion of the workbook, a summary report is generated that we

recommend be included with a manuscript submission to a journal and added to supplementary material when published. This checklist will allow an editor, reviewer, or reader to determine if minimum guidelines were met for lipidomic data presented in a manuscript. This workbook will be available on the LSI website where it will undergo regular updates and revisions based on the needs of the lipidomics field.


a
Overall study design
Lipid extraction
Lipid identification
Quality control
Reporting
Preanalytics/Sample Material
Analytical platform
Lipid Quantification
Method validation
b
Overall study design
Generation
Freeze thaw cycles
Storage temperature
Sample age
Preanalytics
Direct infusion
Separation
Imaging
Quantification type
Lipid extraction
None
Liquid - Liquid
Solid phase
Other
Analytical platform
HR
Lipid identification
Separation
IMS (CCS)
LC (RT)
ESI/Infusion
MS¹ level
Polarity negative
Polarity positive
Isotope correction
Standard verified
Background check
Shorthand nomenclature
--> Lipid rep. level, species
MS² Level
Number of fragments
Type of fragments
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--> Lipid rep. level, species
MS³ Level
Polarity modes
Isotope correction
Standard verified
Shorthand nomenclature
--> Lipid rep. level, molecular
Lipid Quantitation
Quality control
Method validation
Data Reporting
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