Short Communication

Clinical lipidomics – A community-driven roadmap to translate research into clinical applications

Olga Vvedenskaya [a,b], Michal Holčapek [c], Michael Vogeser [d], Kim Ekroos [e], Peter J. Meikle [f], Anne K. Bendt [g,*]

[a] Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany
[b] Spectroswiss Sarl, Lausanne, Switzerland
[c] Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, Pardubice, Czech Republic
[d] Institute for Laboratory Medicine in the Munich University Clinic, Munich, Germany
[e] Lipidomics Consulting Ltd., Esbo, Finland
[f] Baker Heart and Diabetes Institute, Melbourne Victoria, Australia
[g] Singapore Lipidomics Incubator (SLING), Life Sciences Institute, National University of Singapore, Singapore

ARTICLE INFO

ABSTRACT

Lipid metabolites, beyond triglycerides and cholesterol, have been shown to have vast potential for applications in clinical applications, with substantial societal and economical value. To successfully evolve from the current research-grade methods to assays suitable for routine clinical applications, a harmonization – if not standardization – of these mass spectrometry-based workflows is necessary. Input on clinical needs and technological capabilities must be obtained from all relevant stakeholders, including wet lab scientists, informaticians and data scientists, manufacturers, and medical professionals. In order to build bridges between this diverse group of professionals, the International Lipidomics Society and its Clinical Lipidomics Interest Group were created. This opinion article is intended to provide an overview of international efforts to tackle the issues of workflow harmonization, and to serve as an open invitation for others to join this growing community.

1. Introduction

The clinical lipidomics field is steadily growing and addresses a wide range of diseases from monogenic diseases [1] to complex chronic diseases [2–6]. Consequently, the number of research studies, the resulting amount of biomedical data, and available predictive models are increasing year by year (see Fig. 1). If we are to optimize the translational opportunities arising from these growing resources, we require synchronization of efforts to progress the field towards useful clinical applications while minimizing unnecessary work [7]. Notable past and ongoing global efforts by the lipidomics community include the LIPID MAPS initiative, which launched international lipidomics efforts, spearheading lipid classification and nomenclature, databases, standards and other resources for the international community in the nearly 20 years of its existence [8]. The recent £1.3 M Wellcome Trust grant for LIPID MAPS allowed the release of new databases, such as the largest curated lipid structure database, and powerful informatics resources for community use [9].

Concomitantly, in recent decades mass spectrometry-based workflows have become more technically robust and user-friendly, paving the way for adoption by routine clinical laboratories and, hence, potentially substantially expanding the number of lipids used in diagnostics beyond cholesterol and triacylglycerols [10–12]. Another notable effort in this direction is coordinated by the 'Lipidomics Standards Initiative' [13], developing common standards for minimum acceptable data quality and reporting for lipidomics data [14].

Similar initiatives are driven by the Metabolomics Society and its 'metabolite identification' task group, developing and providing standard reporting tools for inter-laboratory comparisons. Their clinical efforts are tackled by two task groups: precision medicine and pharmacometabolomics, and metabolomics epidemiology. The precision medicine group seeks to catalyze the engagement of our metabolomics community in global initiatives in precision medicine [15]. The metabolomics epidemiology group's mission is to promote the growth and understanding of metabolomic epidemiology as an independent research discipline. Such diversification of efforts became possible

---

* Corresponding author.
  E-mail address: anne.bendt@nus.edu.sg (A.K. Bendt).

because metabolomics analyses have been incorporated into routine clinical diagnostics for decades, allowing different research groups to focus on more specific research and clinical topics.

In clinical practice, the next frontier would be to expand from currently single lipid markers to multianalyte lipid panels or lipidomic readouts, enabling a more comprehensive readout of lipid related pathophysiologies, with potential applications in personalized health and precision medicine [2,16–22]. However, these emerging opportunities require interdisciplinary efforts in order to develop workflows with clinical utility, gain wider acceptance and clinical adoption, and ultimately deliver improved outcomes for patients. Currently, the community is fragmented and largely unaware of each other's needs and capacities; experts from different fields speak different languages. To identify the main gaps in our present understanding and capabilities, we need a constructive dialogue between representatives of all fields: lipidomics and biomedical researchers, analytical chemists, representatives of regulatory institutions (i.e., FDA, EMA, CLSI), data scientists, and, of course, clinicians who identify unmet clinical needs.

The *Achilles*' heel of clinical lipidomics is a lack of harmonization and standard reference measurements, a challenge shared with other omics technologies and eloquently discussed in a recent Clinical Chemistry podcast [23].

Harmonization, according to the Cambridge dictionary, is the act of making different people, plans, situations, etc. suitable for each other or so that they can work together more easily. In the case of clinical lipidomics, harmonization is not about prescribing 'the' best method, but, indeed, to understand where the field stands. This could be achieved by using the same materials (i.e., shared reference materials) and reporting minimal quality standards. Standardization, in its turn, is the process of making things of the same type all have the same basic features. In the case of clinical lipidomics, standardization would reflect a much more stringent and less flexible approach towards the standard operating procedures. That said, it is essential to underline that while standardization is seen as essential step towards routine clinical applications, it is outside the scope of the current community efforts and will, once deemed timely, require collaboration with parties, such as the Centers for Disease Control and Prevention, for their laboratory quality assurance and standardization programs.

As an attempt to help coordinate translational efforts in the field of clinical lipidomics, the 'Clinical Lipidomics Interest Group' (CLIG) within the ILS was founded in 2019. Starting with four steering committee members, Michal Holčapek (Czech Republic), Anne K Bendt (Singapore), Michael Vogeser (Germany), and Peter J Meikle (Australia), in less than two years CLIG has grown to about 100 members, representing academia, hospitals, core facilities, industries, such as mass spectrometer manufacturers, service providers and kit developers, and governmental agencies around the world. This opinion article is an open invitation to all interested in clinical translation to join this growing community.

The primary goal of CLIG is to drive the translation of lipidomic research technologies and relevant findings to clinical laboratories for diagnostic and therapeutic purposes. The initial tasks were to: (i) identify bottlenecks in this translation process, (ii) define options to tackle these hurdles, and (iii) serve as a resource for the international community. A roadmap for CLIG activities was developed, outlining necessary community-based activities to advance progress towards the common goal of clinical translation (Fig. 2).

Due to the Covid-19 pandemic, the typical avenue for community-driven initiatives, international conferences, was replaced by monthly meetings, with recordings available to all members. During an online seminar in October 2020, attended by over 150 participants, the main topic of discussion was the challenge of inter-laboratory comparability and reproducibility: analytical workflows applied to analyze lipids in body fluids and tissues are rarely harmonized and often deliver different results [24–26]. This is a particular barrier to the deployment of these methods in clinical settings, as it limits not only trust, but the ability to develop reference ranges for any lipidomic score or test. Further, the results delivered to clinicians and patients need to be correctly determined independent of where or how they were acquired. The planned development of reference ranges for lipid species of clinical interest is primarily driven by another group within ILS, the 'Reference materials and biological reference ranges', working closely with CLIG and the Lipidomics Standards Initiative. This community recently organized ring trials for lipids with demonstrated clinical value: ceramides and bile acids. For ceramides, a small panel of four ceramide species plus their deuterated internal standards were measured by over 30 laboratories
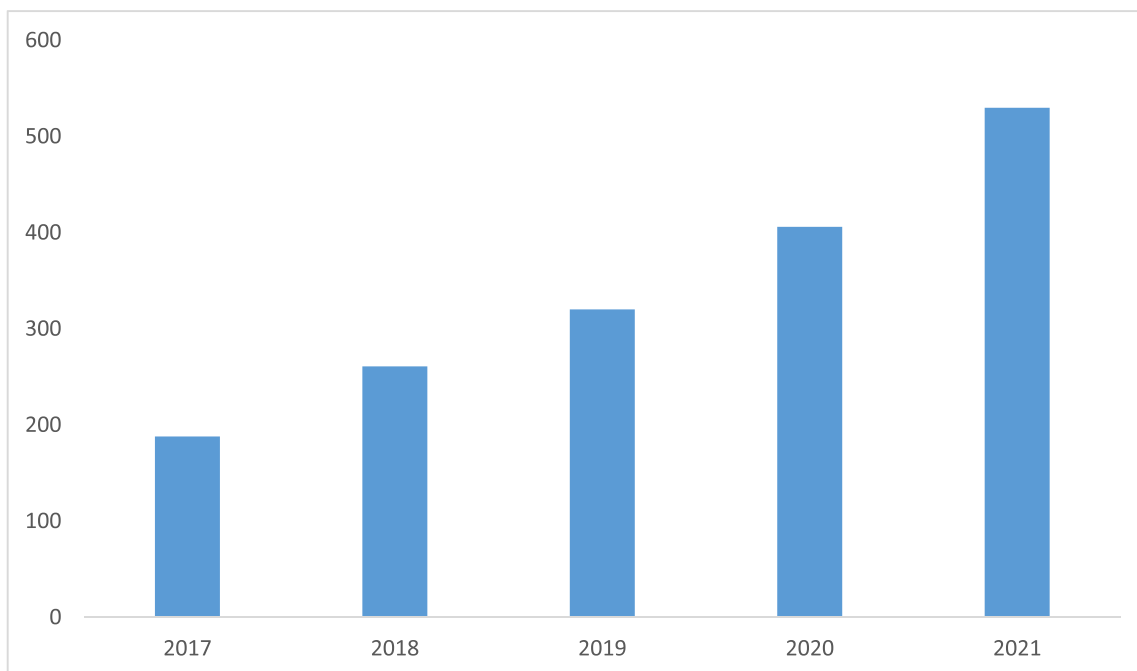

Fig. 1. The number of publications dedicated to clinical lipidomics (data from PubMed obtained on January 05, 2022); search term "clinical lipidomics".
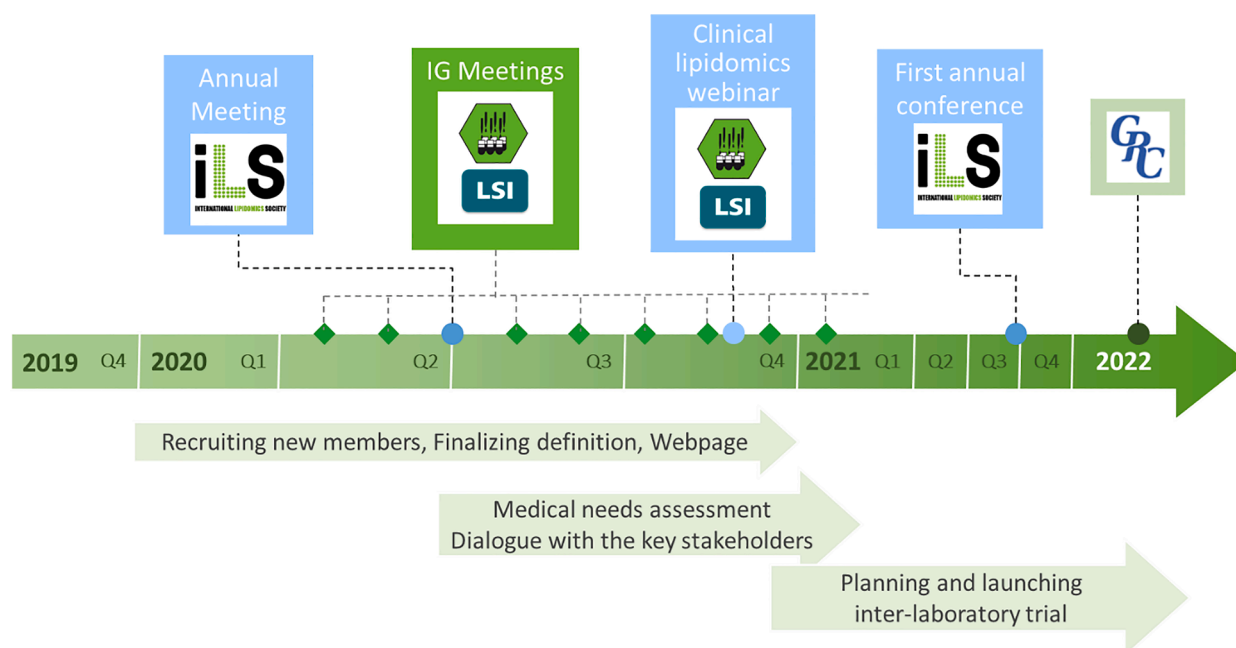
Fig. 2. The community-building roadmap of the Clinical Lipidomics Interest Group. Major events such as conferences and activities including the medical needs assessment and its resulting planning of the inter-laboratory trial are visualized.

worldwide, following a well described protocol and, importantly, using shared reference materials provided by NIST [27]. Once published, the reported molecular concentrations for these four ceramides in human plasma materials will (i) show us how similar values reported by 30 different labs using the same methods can be, and (ii) serve as a basis for the next phase of these international efforts: to quantitate these lipids in a variety of diverse populations, with the final goal of determining their reference values, a prerequisite for clinical diagnostics.

A vast variety of analytical configurations are used for mass spectrometry-based quantitation of lipids in research settings, but simplified and streamlined workflows that are suitable within the realities of routine clinical laboratories are necessary to deliver robust and reliable data. The same issues apply for sample collection and storage: in the absence of commonly agreed upon, harmonized and validated methods, the translation of lipidomic results (i.e., biomarker panels) to the clinic will be challenging. To tackle these issues, the CLIG proposes to establish an international Clinical Lipidomics Platform. In the first instance, this includes an inter-laboratory ring trial, to be launched in 2022. The shared material (human plasma materials) [27] will be analyzed by all participating labs, according to well-described mass spectrometry-based workflows. This technical phase will include analytical method validation according to FDA and EMA guidelines. An optimal outcome would constitute the identification of a lipidomic profile (i.e., multi-analyte panel) that can be reproducibly measured and quantified across laboratories, methodologies, and countries.

In a second phase, CLIG proposes to launch an inter-laboratory biomarker trial to assess the clinical utility of a large lipid panel for clinical use. This effort will be driven by identified unmet clinical needs.

Together, this first 'technical' study followed by the second 'clinical' trial will form the foundation of our overarching goal: to facilitate the translation of lipidomic research findings into routine clinical practice. Incidentally, computational workflows for data analysis of these 'clinical trials' are crucially important for the successful identification and validation of such multi-analyte marker panels. Ideally, computational method evaluation should, hence, be part of future clinical lipidomics ring trials.

Our goals can only be achieved with the effective interplay of diverse stakeholders, collaborating as a team on the various aspects of clinical lipidomics, including pre-analytical, analytical, and post-analytical

challenges. With this opinion paper, we would like to invite medical doctors and clinical laboratory experts, analytical chemists, lipid data scientists, professionals representing regulatory bodies, mass spectrometry specialists, manufacturers, and all individuals interested in clinical lipidomics to join our group. Achievement of CLIG goals have been, and continue to be, only possible thanks to the contributions from our partners, which are listed on our webpage https://lipidomicssociety.org/shop/. We are extremely grateful for their ongoing support of this initiative.

Over the coming years, CLIG will continue to create a quantifiable value for the lipidomics community via various activities. Namely, expanding research and educational resources, organizing webinars, publishing articles, supporting grant applications and getting new research materials tested. To effectively achieve our goals, it is essential to involve various groups at all research and clinical process stages, and include the end users – patients and the medical experts who care for them. We encourage any team or person with interest in clinical lipidomics to join our collaborative project. We are interested in your input, opinion, and expertise. We will support your initiative, so that – together – we can bring innovative ideas and projects to life.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] J.T. Rämö, P. Ripatti, R. Tabassum, S. Söderlund, N. Matikainen, M.J. Gerl, C. Klose, M.A. Surma, N.O. Stitziel, A.S. Havulinna, Coronary artery disease risk and lipidomic profiles are similar in hyperlipidemias with family history and population-ascertained hyperlipidemias, J. Am. Heart Assoc. 8 (13) (2019) e012415.

[2] O. Vvedenskaya, T.D. Rose, O. Knittelfelder, A. Palladini, J.A.H. Wodke, K. Schumann, J.M. Ackerman, Y. Wang, C. Has, M. Brosch, V.R. Thangapandi, S. Buch, T. Züllig, J. Hartler, H.C. Köfeler, C. Röcken, Ü. Coskun, E. Klipp, W. von Schoenfels, J. Gross, C. Schafmayer, J. Hampe, J.K. Pauling, A. Shevchenko, Non-alcoholic fatty liver disease stratification by liver lipidomics, J. Lipid Res. (2021) 100104.

[3] M. Hilvo, P.J. Meikle, E.R. Pedersen, G.S. Tell, I. Dhar, H. Brenner, B. Schöttker, M. Lääperi, D. Kauhanen, K.M. Koistinen, Development and validation of a

ceramide-and phospholipid-based cardiovascular risk estimation score for coronary artery disease patients, Eur. Heart J. 41 (3) (2020) 371–380.

[4] Y.K. Tham, K.S. Jayawardana, Z.H. Alshehry, C. Giles, K. Huynh, A.A.T. Smith, J.Y. Y. Ooi, S. Zoungas, G.S. Hillis, J. Chalmers, Novel lipid species for detecting and predicting atrial fibrillation in patients with type 2 diabetes, Diabetes 70 (1) (2021) 255–261.

[5] K. Huynh, W.L.F. Lim, C. Giles, K.S. Jayawardana, A. Salim, N.A. Mellett, A.A. T. Smith, G. Olshansky, B.G. Drew, P. Chatterjee, I. Martins, S.M. Laws, A.I. Bush, C.C. Rowe, V.L. Villemagne, D. Ames, C.L. Masters, M. Arnold, K. Nho, A.J. Saykin, R. Baillie, X. Han, R. Kaddurah-Daouk, R.N. Martins, P.J. Meikle, Concordant peripheral lipidome signatures in two large clinical studies of Alzheimer's disease, Nat Commun. 11 (1) (2020) 5698.

[6] D. Wolrab, R. Jirásko, M. Chocholoušková, O. Peterka, M. Holčapek, Oncolipidomics: Mass spectrometric quantitation of lipids in cancer research, TrAC, Trends Anal. Chem. 120 (2019).

[7] T.G. Meikle, K. Huynh, C. Giles, P.J. Meikle, Clinical lipidomics: realizing the potential of lipid profiling, J. Lipid Res. (2021) 100127.

[8] V.B. O'Donnell, E.A. Dennis, M.J.O. Wakelam, S. Subramaniam, LIPID MAPS: Serving the next generation of lipid researchers with tools, resources, data, and training, Sci. Signal. 12 (563) (2019) eaaw2964–eaaw2964.

[9] V.B. O'Donnell, K. Ekroos, G. Liebisch, M. Wakelam, Lipidomics: Current state of the art in a fast moving field, Wiley Interdiscip. Rev. Syst. Biol. Med. 12 (1) (2020) e1466.

[10] M. Holcapek, E. Cifkova, M. Lisa, R. Jirasko, D. Wolrab, H. Tereza, A method of diagnosing pancreatic cancer based on lipidomic analysis of a body fluid. EP3514545B1, 2020.

[11] M. Bitenc, K. Kruusmaa, P. Hurtado-Castillo, A.M. Jiménez-Girón, R. Argamasilla-Martinez, A. Fabregat-Rossel, A.J. Adsuar-Gomez, J. Martinez-Barea, C. Hense, P. Rodríguez-Gómez, Á. Peralbo-Molina, J. Casado-Agrelo, A. Sánchez-Brotons, C. Pavón-Solís, R.M. Delgado-Sánchez, Methods and systems for metabolite and/or lipid-based detection of colorectal cancer and/or adenomatous polyps. 2018.

[12] 12. Reijo L, Ekroos K, Reini H, Katainen R. Lipidomic biomarkers for identification of high-risk coronary artery disease patients. 10955427, 2021.

[13] Lipidomics-Standards-Initiative (LSI). Lipidomics Standards Initiative [Internet]. Available from: https://lipidomics-standards-initiative.org.

[14] G. Liebisch, R. Ahrends, M. Arita, M. Arita, J.A. Bowden, C.S. Ejsing, W.J. Griffiths, M. Holčapek, H. Köfeler, T.W. Mitchell, M.R. Wenk, K. Ekroos, Consortium LSI, Lipidomics needs more standardization, Nat. Metab. 1 (8) (2019) 745–747, https://doi.org/10.1038/s42255-019-0094-z.

[15] Metabolomics Society I. Metabolomics Society, Scientific Task Groups [Internet]. Available from: https://metabolomicssociety.org/board-committees/scientific-task-groups/.

[16] N. Köhler, T.D. Rose, L. Falk, J.K. Pauling, Investigating global lipidome alterations with the lipid network explorer, Metabolites 11 (8) (2021) 488.

[17] H. Tsugawa, K. Ikeda, M. Arita, The importance of bioinformatics for connecting data-driven lipidomics and biological insights, Biochim. Biophys. Acta, Mol. Cell. Biol. Lipids 1862 (8) (2017) 762–765.

[18] S. Sales, J. Graessler, S. Ciucci, R. Al-Atrib, T. Vihervaara, K. Schuhmann, D. Kauhanen, M. Sysi-Aho, S.R. Bornstein, M. Bickle, C.V. Cannistraci, K. Ekroos,

[19] A. Shevchenko, Gender, contraceptives and individual metabolic predisposition shape a healthy plasma lipidome, Sci. Rep. 6 (1) (2016) 1–14.

[19] Y.A. Ambaw, M.P. Pagac, A.S. Irudayaswamy, M. Raida, A.K. Bendt, F.T. Torta, M. R. Wenk, T.L. Dawson, Host/malassezia interaction: A quantitative, non-invasive method profiling oxylipin production associates human skin eicosanoids with malassezia, Metabolites 11 (10) (2021) 700.

[20] Y. Nakashima, Y. Sakai, Y. Mizuno, K. Furuno, K. Hirono, S. Takatsuki, H. Suzuki, Y. Onouchi, T. Kobayashi, K. Tanabe, Lipidomics links oxidized phosphatidylcholines and coronary arteritis in Kawasaki disease, Cardiovasc. Res. 117 (1) (2021) 96–108.

[21] T. Hirata, K. Yamamoto, K. Ikeda, M. Arita, Functional lipidomics of vascular endothelial cells in response to laminar shear stress, FASEB J. 35 (2) (2021).

[22] D. Wolrab, R. Jirásko, E. Cífková, M. Höring, D. Mei, M. Chocholoušková, O. Peterka, J. Idkowiak, T. Hrnčiarová, L. Kuchař, R. Ahrends, R. Brumarová, D. Friedecký, G. Vivo-Truyols, P. Škrha, J. Škrha, R. Kučera, B. Melichar, G. Liebisch, R. Burkhardt, M.R. Wenk, A. Cazenave-Gassiot, P. Karásek, I. Novotný, K. Greplová, R. Hrstka, M. Holčapek, Lipidomic profiling of human serum enables detection of pancreatic cancer, Nat. Commun. 13 (1) (2022) 124.

[23] B.A. Rappold, A. Hoofnagle, Clinical Chemistry Podcast: Clinical Protein Analysis by Mass Spectrometry: A New Higher Order.

[24] J.A. Bowden, A. Heckert, C.Z. Ulmer, C.M. Jones, J.P. Koelmel, L. Abdullah, L. Ahonen, Y. Alnouti, A.M. Armando, J.M. Asara, T. Bamba, J.R. Barr, J. Bergquist, C.H. Borchers, J. Brandsma, S.B. Breitkopf, T. Cajka, A. Cazenave-Gassiot, A. Checa, M.A. Cinel, R.A. Colas, S. Cremers, E.A. Dennis, J.E. Evans, A. Fauland, O. Fiehn, M.S. Gardner, T.J. Garrett, K.H. Gotlinger, J. Han, Y. Huang, A.H. Neo, T. Hyötyläinen, Y. Izumi, H. Jiang, H. Jiang, J. Jiang, M. Kachman, R. Kiyonami, K. Klavins, C. Klose, H.C. Köfeler, J. Kolmert, T. Koal, G. Koster, Z. Kuklenyik, I. J. Kurland, M. Leadley, K. Lin, K.R. Maddipati, D. McDougall, P.J. Meikle, N. A. Mellett, C. Monnin, M.A. Moseley, R. Nandakumar, M. Oresic, R. Patterson, D. Peake, J.S. Pierce, M. Post, A.D. Postle, R. Pugh, Y. Qiu, O. Quehenberger, P. Ramrup, J. Rees, B. Rembiesa, D. Reynaud, M.R. Roth, S. Sales, K. Schuhmann, M.L. Schwartzman, C.N. Serhan, A. Shevchenko, S.E. Somerville, L. St John-Williams, M.A. Surma, H. Takeda, R. Thakare, J.W. Thompson, F. Torta, A. Triebl, M. Trötzmüller, S.J.K. Ubhayasekera, D. Vuckovic, J.M. Weir, R. Welti, M.R. Wenk, C.E. Wheelock, L. Yao, X.H. Yuan, X.H. Zhao, S. Zhou, Harmonizing lipidomics: NIST interlaboratory comparison exercise for lipidomics using SRM 1950-Metabolites in frozen human plasma, J. Lipid Res. 58 (12) (2017) 2275–2288.

[25] B. Burla, M. Arita, M. Arita, A.K. Bendt, A. Cazenave-Gassiot, E.A. Dennis, K. Ekroos, X. Han, K. Ikeda, G. Liebisch, M.K. Lin, T.P. Loh, P.J. Meikle, M. Orešič, O. Quehenberger, A. Shevchenko, F. Torta, M.J.O. Wakelam, C.E. Wheelock, M. R. Wenk, MS-based lipidomics of human blood plasma - a community-initiated position paper to develop accepted guidelines, Available from, J. Lipid Res. (2018), S087163, http://www.jlr.org/lookup/doi/10.1194/jlr.S087163.

[26] O. Vvedenskaya, Y. Wang, J.M. Ackerman, O. Knittelfelder, A. Shevchenko, Analytical challenges in human plasma lipidomics: a winding path towards the truth, TrAC - Trends Anal Chem. 120 (2019).

[27] J.J. Aristizabal-Henao, C.M. Jones, K.A. Lippa, J.A. Bowden, Nontargeted lipidomics of novel human plasma reference materials: hypertriglyceridemic, diabetic, and African-American, Anal. Bioanal. Chem. 412 (27) (2020) 7373–7380.