Title
Harmonizing lipidomics: NIST interlaboratory comparison exercise for lipidomics using SRM 1950-Metabolites in Frozen Human Plasma.

Permalink
https://escholarship.org/uc/item/9gf004j8

Journal
Journal of lipid research, 58(12)

ISSN
0022-2275

Authors
Bowden, John A
Heckert, Alan
Ulmer, Candice Z
et al.

Publication Date
2017-12-01

DOI
10.1194/jlr.m079012

Peer reviewed

Harmonizing lipidomics: NIST interlaboratory comparison exercise for lipidomics using SRM 1950–Metabolites in Frozen Human Plasma

John A. Bowden,1,8 Alan Heckert,9 Candice Z. Ulmer,8 Christina M. Jones,8 Jeremy P. Koelmel,8 Laila Abdullah,1 Linda Ahonen,1 Yazen Alnouti,1 Aaron M. Armando,1 John M. Asara,1,7 Takeshi Bamba,88 John R. Barr,19 Jonas Bergquist,19 Christoph H. Borchers,88 Joost Brandsma,88 Susanne B. Breitkopf,42 Tomas Cajka,42 Amaury Cazenave-Gassiot,41 Antonio Checa,39 Michelle A. Cinel,35 Romain A. Colas,44,45 Serge Cremerens,1111 Edward A. Dennis,5 James E. Evans,1 Alexander Fauland,88 Oliver Fiehn,77,1111 Michael S. Gardner,42 Timothy J. Garrett,8 Katherine H. Godlinger,15,14 Jun Han,18,19 Yingying Huang,17 Tuulia Hyötyläinen,17 Yoshiiro Izumi,18 Hongfeng Jiang,111 Houli Jiang,1111 Jiang Jiang,5,7 Maureen Kachman,3966 Reiko Kiyonami,18 Michael A. Kobayashi,55 Kristaps Klavins,1111 Christian Klose,1111 Harald C. Köfeler,88 Johann Kolmert,88 Therese Koal,44 Grielo Koster,88 Zsuzsanna Kuklenyik,11 Irwin J. Kurland,1111 Michael Leadley,1,11 Karen Lin,18,19 Krishna Rao Maddipati,40 Danielle McDonnell,18,43 Peter J. Meikle,18,55 Natalie A. Mellett,88 Cian Monnin,55 M. Arthur Moseley,88 Renu Nandakumar,1111 Matej Oresic,21 Rainey Patterson,6 David Peake,19 Jason S. Pierce,1111 Martin Post,1111 Anthony D. Postle,88 Rebecca Pugh,88 Yumping Qiu,41,42,43,2021,1111 Oswald Quehenberger,41,42,43,2021 Parsram Ramrup,41,42,43,2021 Jon Rees,18 Barbara Rembriesa,1111 Denis Reynaud,1111 Mary R. Roth,1111 Susanne Sales,1 Kai Schuhmann,18,55,1111 Michal Lianodi Schwartzman,1111 Charles N. Serhan,1111 Andrej Shevchenko,18,55,1111 Stephen E. Somerville,1111 Lisa St. John-Williams,1111 Michal A. Surma,1111 Hiroaki Takeda,39 Rishikesh Thakare,39 J. Will Thompson,1111 Federico Torta,1111 Alexander Triebl,18,55 Martin Trötzmüller,18,55 S. J. Kumari Ubbayasekara,18 Dajana Vuckovic,41,42,43 Lacelyn M. Weir,1111 Ruth Welti,1111 Markus R. Wenk,1111 Craig E. Wheelock,41 Libin Yao,41 Min Yuan,18 Xueqing Heather Zhao,1111 and Senlin Zhou

Marine Biochemical Sciences Group,6 Chemical Sciences Division, Hollings Marine Laboratory, National Institute of Standards and Technology, Charleston, SC; Statistical Engineering Division,1 National Institute of Standards and Technology, Gaithersburg, MD; Department of Pathology, Immunology, and Laboratory Medicine,8 University of Florida, Gainesville, FL; The Roskamp Institute,2 Sarasota, FL; Steno Diabetes Center Copenhagen,1 Gentofte, Denmark; Department of Pharmaceutical Sciences,9 University of Nebraska Medical Center, Omaha, NE; Departments of Chemistry and Biochemistry and Pharmacology,5 School of Medicine, University of California, San Diego, La Jolla, CA; Division of Signal Transduction,9,9 Beth Israel Deaconess Medical Center, Boston, MA; Department of Medicine,11 Harvard Medical School, Boston, MA; Division of Metabolomics,19 Research Center for Transomics Medicine, Medical Institute of Bioregulation, Kyushu University, Higashi-ku, Fukuoka, Japan; Division of Laboratory Sciences,11 Centers for Disease Control and Prevention, National Center for Environmental Health, Atlanta, GA; Department of Chemistry-BMC,5 Analytical Chemistry, Uppsala University, Uppsala, Sweden; University of Victoria-Genome British Columbia Proteomics Centre19 and Department of Biochemistry and Microbiology,39 University of Victoria, Victoria, British Columbia, Canada; Gerald Bronfman Department of Oncology*** and Proteomics Centre, Segal Cancer Centre, Lady Davis Institute,1111 Jewish General Hospital, McGill University, Montreal, Quebec, Canada; Faculty of Medicine,355 Academic Unit of Clinical and Experimental Sciences, Southampton General Hospital, University of Southampton, Southampton, United Kingdom; National Institutes of Health West Coast Metabolomics Center,1 University of California Davis Genome Center, Davis, CA; Department of Biochemistry,9 Yong Loo Lin School of Medicine, National University of Singapore and Singapore Lipidomic Incubator (SLING), Life Sciences Institute, Singapore; Division of Physiological Chemistry 2,1211 Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden; Baker Heart and Diabetes Institute,355 Melbourne, Victoria, Australia; Department of Anesthesiology, Perioperative and Pain Medicine,21 Center for Experimental Therapeutics and Reperfusion Injury, Brigham and Women’s Hospital and Harvard Medical School, Boston, MA; Biomarker Core Laboratory,1111 Irving Institute for Clinical and Translational Research, Columbia University Medical Center, New York, NY; Biochemistry Department,88 King Abdulaziz University, Jeddah, Saudi Arabia; Department of Pharmacology,1111 New York Medical College School of Medicine, Valhalla, NY; Thermo Fisher Scientific,10 San Jose, CA; Department of Chemistry,355 Örebro University, Örebro, Sweden; Metabolomics Core,355 BRCF, University of Michigan,
Abstract As the lipidomics field continues to advance, self-evaluation within the community is critical. Here, we performed an interlaboratory comparison exercise for lipidomics using Standard Reference Material (SRM) 1950—Metabolites in Frozen Human Plasma, a commercially available reference material. The interlaboratory study comprised 31 diverse laboratories, with each laboratory using a different lipidomics workflow. A total of 1,527 unique lipids were measured across all laboratories and consensus location estimates and associated uncertainties were determined for 339 of these lipids measured at the sum composition level by five or more participating laboratories. These evaluated lipids detected in SRM 1950 serve as community-wide benchmarks for intra- and interlaboratory quality control and method validation. These analyses were performed using nonstandardized laboratory-independent workflows. The consensus locations were also compared with a previous examination of SRM 1950 by the LIPID MAPS consortium. While the central theme of the interlaboratory study was to provide values to help harmonize lipids, lipid mediators, and precursor measurements across the community, it was also initiated to stimulate a discussion regarding areas in need of improvement.

Abbreviations: BA, bile acid; CE, cholesteryl ester; CER, ceramide; COA, certificate of analysis; COD, coefficient of dispersion; CRM, certified reference material; DAG, diacylglycerol; DLS, DerSimonian-Laird; FC, free cholesterol; GL, glycerolipid; GP, glycerophospholipid; Hex-Cer, hexosylceramide; IR, internal report; LIPID MAPS, LIPID Metabolites and Pathways Strategy; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; MEDM, median of means; NIST, National Institute of Standards and Technology; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; SP, sphingolipid; SRM, Standard Reference Material; ST, sterol; TAG, triacylglycerol.

To whom correspondence should be addressed. e-mail: john.bowden@nist.gov

The online version of this article (available at http://www.jlr.org) contains a supplement.
The relationship between lipids and human health has been explored as early as the 1900s, where lipids were noted as important nutritional factors (1, 2) and were frequently found to be altered from homeostatic concentrations in pathophysiological conditions (3–5). Throughout the century, lipids have been increasingly used to evaluate human health. However, it was not until the early 2000s, with the advent of mass spectrometric approaches (6, 7), that the potential of lipid research could be realized. With the increased capacity to interrogate the lipidome, the number and types of human health applications employing lipid analysis have steadily risen (8–11). Over this period of rapid advancement, the lipidomics community, with leading endeavors from LIPID Metabolites and Pathways Strategy (LIPID MAPS), has pursued efforts to characterize several lipidomes, improve quantitative measurements, and delineate the complex milieu of lipid interactions and pathways (12, 13). In 2010, LIPID MAPS formed a consortium to define the constituents of the mammalian lipidome using the National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 1950–Metabolites in Frozen Human Plasma (14). The resulting lipidome was earmarked at 588 lipid species above error thresholds. This concerted effort was achieved piecemeal by separate core laboratories via contributions predominantly employing triple quadrupole MS technology for targeted lipid class measurements.

Within the past 5 years, advances in chromatography and the advent of high-resolution MS have resulted in the measurement of a greater spectrum of lipids within the lipidome using a single platform (15–17). With this enhanced coverage of the lipidome, there is an increased probability of characterizing lipid pathways perturbed by disease. This is supported by the dramatic increase in potential biomarker discovery applications in lipidomics using untargeted platforms (18, 19). However, as the lipidomics field expands from targeted assays, using predominantly triple quadrupole technology, to untargeted and perhaps back to targeted assays, using state of the art technology across a diverse range of workflows and platforms, it is important for the lipidomics community to monitor and improve measurement activities.

The same inherent qualities that lend themselves to the maturation of lipidomics and its widespread use as an approach to examine human health, namely the vast complexity in lipid structure, function, and abundance and their ubiquitous existence at membrane, cellular, tissue, and systemic levels (20, 21), also imbue a variety of measurement challenges. Despite these challenges, lipidomic studies continue to emerge at an increased rate and with a push toward precision medicine (22–26). However, a substantial roadblock in the progression of translating lipidomics from the bench to routine clinical settings is the lack of standardization or harmonization within the lipidomics community (27). Without standardization, the assessment of data quality independent of time, place, and procedure is difficult (28, 29). As the field of lipidomics continues to progress, it will be critical to be able to control, minimize, or, at the very least, understand intra- and interlaboratory variability to ensure confidence in the discovery of real biological differences (30, 31). Several excellent lipidomics reviews (15, 32–34) conclude that the differences in methodology within the lipidomics community are extensive. This variation in lipidomics methodology has a direct impact on the resultant lipid profiles observed, affecting the number, type, and quantity of lipids observed (30, 31, 35). To date, the exact impact of this methodological diversity on community-wide lipid measurement and agreement is unknown.

Interlaboratory studies, where participants are instructed to perform a specific analysis on a homogenous and stable reference material followed by an evaluation and comparison of data at both an intra- and interlaboratory level, are exercises well-suited to critically evaluate the agreement of measurement within the lipidomics community and highlight areas of concern. NIST and others have coordinated interlaboratory studies across disciplines for a wide variety of analytes, including omics-based profiles (36–43). For the latter, specifically for proteomics and metabolomics, interlaboratory studies have been presented with the theme of addressing the lack of agreement within the community by highlighting the need to develop standards, guidelines, and protocols, and to identify ways to evaluate laboratory performance, quality control, and dissemination (43–46). The paucity of commercially available reference materials for lipidomics and the lack of a reason to extend quality control practices beyond the intralaboratory level have limited the ability to benchmark data within the lipidomics community. The use of SRM 1950 as a control material for small molecule-based omics studies continue to emerge at an increased rate and with a push toward precision medicine (22–26). However, a substantial roadblock in the progression of translating lipidomics from the bench to routine clinical settings is the lack of standardization or harmonization within the lipidomics community (27). Without standardization, the assessment of data quality independent of time, place, and procedure is difficult (28, 29). As the field of lipidomics continues to progress, it will be critical to be able to control, minimize, or, at the very least, understand intra- and interlaboratory variability to ensure confidence in the discovery of real biological differences (30, 31). Several excellent lipidomics reviews (15, 32–34) conclude that the differences in methodology within the lipidomics community are extensive. This variation in lipidomics methodology has a direct impact on the resultant lipid profiles observed, affecting the number, type, and quantity of lipids observed (30, 31, 35). To date, the exact impact of this methodological diversity on community-wide lipid measurement and agreement is unknown.

Interlaboratory studies, where participants are instructed to perform a specific analysis on a homogenous and stable reference material followed by an evaluation and comparison of data at both an intra- and interlaboratory level, are exercises well-suited to critically evaluate the agreement of measurement within the lipidomics community and highlight areas of concern. NIST and others have coordinated interlaboratory studies across disciplines for a wide variety of analytes, including omics-based profiles (36–43). For the latter, specifically for proteomics and metabolomics, interlaboratory studies have been presented with the theme of addressing the lack of agreement within the community by highlighting the need to develop standards, guidelines, and protocols, and to identify ways to evaluate laboratory performance, quality control, and dissemination (43–46). The paucity of commercially available reference materials for lipidomics and the lack of a reason to extend quality control practices beyond the intralaboratory level have limited the ability to benchmark data within the lipidomics community. The use of SRM 1950 as a control material for small molecule-based omics studies has been supported by a recent white paper on metabolomics-enabled precision medicine (47), where it is recommended that this certified reference material (CRM) be used as a material to aid in standardization and quality assessment across time and laboratories, at least until new reference materials are created. NIST produced this commercially available homogeneous material to aid in standardizing clinical measurements; other reports have noted its potential as a metabolomics reference material (14, 48–52). We propose that SRM 1950

Supplementary key words: fatty acyls • glycerolipids • lipids • phospholipids • quality control • quantitation • sphingolipids • Standard Reference Material 1950 • sterols • National Institute of Standards and Technology

Interlaboratory comparison exercise for lipidomics using SRM 1950—Metabolites in Frozen Human Plasma. J. Lipid Res. 2017. 58: 2275–2288.

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. Quechenberger, 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. Tribel, M. Trötzmüller, S. J. K. Ubhayasekera, D. Vuckovic, J. M. Weir, R. Welti, M. R. Wenk, C. E. Wheelock, L. Yao, M. Yuan, X. H. Zhao, and S. Zhou. Harmonizing lipidomics: NIST interlaboratory comparison exercise for lipidomics using SRM 1950—Metabolites in Frozen Human Plasma. J. Lipid Res. 2017. 58: 2275–2288.
has equal value as a quality control sample for lipidomics and, thus, would be a suitable material for an interlaboratory comparison exercise.

Since 2014, NIST has been conducting an interlaboratory comparison exercise for lipidomics using SRM 1950. To provide a true cross-section of the lipidomics community, 31 national and international laboratories, composed of both global and targeted lipidomic methodologies spanning across academia, industry, and core facilities, have participated. The interlaboratory study was designed to highlight: 1) the extent of agreement present in current lipidomic measurement within the community, 2) determine consensus locations with associated uncertainties for lipids present in SRM 1950, and 3) highlight the challenges present in current lipid measurements in regard to lipid methodology employed. In this work, we address the first two goals above; while a follow-up work will address methodologies used and the effect on quantitation. Reference results have been established for 359 lipids present in SRM 1950 that can be used by laboratories to assess whether their data agree with the lipidomics community. These consensus locations are compared with the concentration values noted from the LIPID MAPS consortium (14).

MATERIALS AND METHODS

SRM 1950

A vial of SRM 1950–Metabolites in Frozen Human Plasma was shipped on dry ice to participating laboratories. In collaboration with the National Institute of Diabetes and Digestive and Kidney Diseases, NIST developed SRM 1950 in 2006 as a "normal" human plasma reference material. A full description of this material is provided in its certificate of analysis (COA) (www.nist.gov/srm). In brief, this plasma material was constructed from 100 fasted individuals in the age range of 40–50 years, who represented the average composition of the US population, as defined by race, sex, and health (extreme health cohorts were excluded) (53). Due to these factors and its commercial availability, this material was selected for use in the interlaboratory lipidomics comparison exercise.

Overview of exercise

Participants in the exercise were provided a data submission template that contained several tabs focused on obtaining basic laboratory and method information: sample preparation, sample introduction and chromatography, mass spectrometric approach, and data processing. Unless the participant declined to disclose details, information was obtained on sample chain of custody, extraction methodology, internal standard selection, chromatographic methods, mass spectrometer type, scanning approach employed (global and/or targeted), and the data handling/software utilized. For the analysis of SRM 1950, each laboratory was asked to employ the analytical procedures traditionally used in their laboratories and to report lipids identified and quantified (in triplicate) at nanomoles per milliliter of plasma concentration levels. Laboratories were informed that all information, which could be used to link laboratories to their submitted data, would be excluded in the resulting publications.

The template, which also listed potential target lipid species, is reproduced in NIST Internal Report (IR) 8185 (54).

Organization of submitted data

Each participating laboratory submitted an Excel workbook that contained lipid identifications and the respective triplicate concentration measurements (nanomoles per milliliter). Upon receipt of data, the mean and standard deviation were calculated for lipids with three replicates and nonzero concentrations. Submitted data entries (lipid species name, m/z reported, and the adduct utilized) were compared with LipidPioneer (55) for accuracy and consistency. Specifically, LipidPioneer was used to calculate the m/z of various adducts observed given the lipid name. Features were flagged and researchers contacted if discrepancies were observed between the lipid name and the m/z reported. Submission errors found in lipid species assignment, mass assignment, and/or adduct reported were edited and subsequently verified by the laboratory. Laboratories reported lipids by fatty acyl constituents and/or by the sum composition [total carbon:total double bonds, (C:DB)] according to the shorthand nomenclature proposed by the International Lipid Classification and Nomenclature Committee (56). All entries were converted to sum composition for comparison across all laboratories. To accomplish this, concentrations for isomer lipid species per replicate were summed and the three replicate sums were used to calculate the mean and standard deviation. As an example, each replicate concentration of phosphatidylcholine PC(16:1_18:1) and PC(16:0_18:2) was summed and reported as PC(34:2). Lipid isomers were included in the summation if they were reported by at least two laboratories.

Calculation of final consensus locations and uncertainties

The concept of calculating a consensus value and its associated uncertainty for measurements from multiple laboratories has been well-studied and there are many approaches available to address this challenge (57). We considered several methods for estimating the consensus location and associated uncertainty for each submitted lipid species. The consensus approach employed for this exercise was the median of means (MEDM) method (58). The MEDM consensus value (“location”) is simply the median of laboratory means. An associated standard uncertainty for the MEDM consensus value, $u$, is $\sqrt{\pi/2m} \times 1.483 \times MAD$, where $m$ and MAD denote the number of laboratories and the median absolute deviation of the laboratory means, respectively (58). Analogous to the sample coefficient of variation, the sample coefficient of dispersion (COD) (59), expressed as a percentage, was calculated as $100 \times u$/MEDM for each lipid species. These COD values were used to facilitate evaluation of the quality or “usefulness” of the consensus estimates. For evaluation purposes, the MEDMs were deemed acceptable for quality control activities if they had a COD value less than 40%.

The data in this study contained several extreme outliers (laboratory mean lipid concentrations). These outliers violated the normality assumptions made by more statistically efficient consensus estimation methods, such as Vangel-Rukhin (60, 61) and DerSimonian-Laird (DSL) (62)). The presence of these outliers resulted in unrepresentative consensus values for these two methods. However, the MEDM method generated reasonable and representative consensus locations without requiring the omission of outlier laboratories from the analysis.

MEDM location estimates (nanomoles per milliliter) are only reported for lipids that were measured by at least five laboratories. NIST IR 8185 (54) details the consensus estimates and uncertainties in both tabular and graphical formats.

Final consensus location comparison

The final consensus location estimates and the associated uncertainties determined in this study were compared with the lipid...
Concentrations noted previously in the analysis of SRM 1950 conducted by the LIPID MAPS consortium (14) using predominantly triple quadrupole technology for targeted lipid class measurements. A percent change was calculated for lipids in SRM 1950, comparing the MEDM calculated in this study to the previously published values of the LIPID MAPS consortium. The values obtained from the LIPID MAPS consortium were set as the reference values in the percent change calculation. The final MEDM lipid species were summed by class to reflect those lipids that were common to the LIPID MAPS consortium.

RESULTS AND DISCUSSION

Construction of the interlaboratory comparison exercise

Lipid measurements were obtained from a diverse collection of laboratories that represent the current cross-section of lipid measurement within the community. Invitations were sent to 100 potential participants, spanning laboratories with differing levels of experience, publication history, and lipid methodology. Of these, 31 laboratories submitted lipidomic data with one laboratory submitting two lipidomic data sets from different MS platforms. The participants consisted of 55% US/45% international-based, 52% global/48% targeted profiling, and 78% academic/22% commercial laboratories (representing industry and government entities). Global profiling laboratories are here defined as those laboratories reporting at least three lipid categories within a data submission. Targeted profiling laboratories are defined here as those laboratories reporting values for lipids in less than three lipid categories. Lipid categories are classified as fatty acyls (FAs), glycerolipids (GLs), glycerophospholipids (GPs), polyketides, prenol lipids, saccharolipids, sphingolipids (SPs), and sterols (STs) (63, 64).

Interlaboratory breakdown of the SRM 1950 plasma lipidome

Since the inception of lipidomics, there have been numerous reports aimed at ascertaining the composition of the human plasma lipidome. Based on the degree of lipid identification (sum composition vs. individual isomers), it has been reported that anywhere between 150 and 700 lipids could be present within the human plasma lipidome (14, 65–72). As lipidomic techniques advance, it is possible that many more lipids will be identified. The LIPID MAPS report on SRM 1950 in 2011, for example, employing targeted class-specific analyses, noted 588 lipid species. At the sum composition level, 1,527 unique lipid identifications were reported in the current study. This value should be viewed conservatively, as it includes the sum of several isomeric lipid species. A breakdown of the lipid species reported, by lipid class, subclass, and number of laboratories reporting, can be found in NIST IR 8185 (54). The 1,527 lipid species represent five lipid categories: FAs (n = 177), GLs (n = 317), GPs (n = 679), SPs (n = 236), and STs (n = 118).

Due to a high incidence of over-reporting observed within the study, lipid species were included in the final MEDM analysis only if reported by at least five laboratories (e.g., 745 lipids identified at the sum composition level were reported by only one laboratory). In total, there were 339 lipids that were reported by ≥5 laboratories: FAs (n = 14), GLs (n = 83), GPs (n = 150), SPs (n = 58), and STs (n = 34). A dissection of the number of lipids by class for those lipids with MEDM values is shown in Fig. 1A. The final calculated MEDM with CODs ≤40% (n = 254) represent the most probable interval for which the true concentration value resides in SRM 1950, especially after factoring in the diverse methodologies employed by participating laboratories. It should be noted that the participating laboratories applied independent protocols in this exercise and, henceforth, did not align their acquisition parameters, extraction protocols, or workflows in assessing the sample. While all laboratories employed different workflows, trends between MEDM location and COD and the number of laboratories reporting and COD were observed. The top-50 most concentrated lipids with MEDM locations had an average COD of 26 ± 11% and were measured by an average...
of 15 ± 4 laboratories. Conversely, the bottom-50 least concentrated lipids with MEDM locations had an average COD of 35 ± 19% and were measured by an average of 7 ± 2 laboratories. The COD values for the top-50 lipids, by concentration, were significantly lower (*P < 0.005, two-sided *t* test) than the bottom-50 lipids. In addition, the number of laboratories reporting for a given lipid species was inversely proportional to the COD, as expected [see (54) for additional details].

Breakdowns of the consensus estimates organized by lipid category are presented for FAs (Table 1), GLs (Table 2), GPs (Table 3), SPs (Table 4), and STs (Table 5). The top five lipid classes using COD < 40% criterion are: triacylglycerols (TAGs) (n = 42), PCs (n = 53), SMs (n = 30), phosphatidylethanolamines (PEs) (n = 29), and lysophosphatidylcholines (LPCs) (n = 25). All major lipid classes are represented (Fig. 2). We endorse these consensus locations for use in quality control.

There were 97 lipids with COD ≤ 20%, representing several lipid classes including: bile acids (BAs) (n = 6), cholesterol (FC), FFAs (n = 2), LPCs (n = 13), PCs (n = 30), PEs (n = 12), phosphatidylinositol (PIs) (n = 12), SMs (n = 6), and TAGs (n = 5). These data suggest that the community measures phospholipids more consistently (specifically LPC, PC, PE, and PI species) relative to other lipid categories. Approximately, 52, 48, 34, and 80% of the LPC, PC, PE, and PI species, respectively, were measured with a COD ≤ 20%. However, for several of the lipids in the LPC class, even though the 40% COD criterion was satisfied, a significant number of laboratory means fell outside ±2 times the standard error of the consensus location estimate. Although this can be explained by noting that the uncertainty for the MEDM method is controlled by the 25% of the laboratory means both above and below the final MEDM estimate, some caution is warranted in using lipids from this class for quality control purposes.

There were 85 lipids with MEDM estimates associated with COD < 40% (supplemental Tables S1–S5 for lipid categories FA, GL, GP, SP, and ST, respectively) in 13 lipid classes: CEs (n = 4), CERs (n = 7), FFAs (n = 6), DAGs (n = 19), diacylglycerols (DAGs) (n = 1), eicosanoids (n = 1), free cholesterol (FC), FFAs (n = 2), LPCs (n = 13), PCs (n = 30), PEs (n = 12), phosphatidylisotol (PIs) (n = 12), SMs (n = 6), and TAGs (n = 5). These data suggest that the community measures phospholipids more consistently (specifically LPC, PC, PE, and PI species) relative to other lipid categories. Approximately, 52, 48, 34, and 80% of the LPC, PC, PE, and PI species, respectively, were measured with a COD ≤ 20%. However, for several of the lipids in the LPC class, even though the 40% COD criterion was satisfied, a significant number of laboratory means fell outside ±2 times the standard error of the consensus location estimate. Although this can be explained by noting that the uncertainty for the MEDM method is controlled by the 25% of the laboratory means both above and below the final MEDM estimate, some caution is warranted in using lipids from this class for quality control purposes.

There were 62 lipids that fit this criterion (supplemental data (62)). For inclusion as a tentative location, we set the difference between the DSL and MEDM estimates equal to 20%. However, for several of the lipids in the LPC class, even though the 40% COD criterion was satisfied, a significant number of laboratory means fell outside ±2 times the standard error of the consensus location estimate. Although this can be explained by noting that the uncertainty for the MEDM method is controlled by the 25% of the laboratory means both above and below the final MEDM estimate, some caution is warranted in using lipids from this class for quality control purposes.

The classes with the greatest percentage of lipids with COD > 40% were CERs (40%), DAGs (79%), FFAs (54%), and TAGs (28%). These findings lend greater insight into the lipids and lipid classes most affected by measurement diversity and emphasize a need to improve measurement uniformity. The lipids with COD > 40% should not be used for quality control; rather, we suggest that these lipids and lipid classes represent challenges requiring improvement in lipid measurement.

By lipid class, the largest overall lipid concentration using the lipids having MEDM values was attributed to CEs (47%), PCs (18%), cholesterol (12%), TAGs (9%), and SMs (5%), as shown in Fig. 1B. The lipid category with the fewest MEDM values was the fatty acyls, which comprised FFAs (n = 11) and eicosanoids (n = 3), as shown in Table 1. As part of this exercise, SRM 1950 was sent to nine targeted laboratories for eicosanoid measurement. Eicosanoids are defined here as lipid mediator analogs produced from polyunsaturated fatty acids. Only six laboratories provided eicosanoid concentrations (two laboratories were not able to measure any eicosanoids in SRM 1950, one laboratory failed to respond). In total, 143 eicosanoids were measured by at least one laboratory; however, only three (5-HETE, 12-HETE, and 15-HETE) were measured by at least five laboratories.

Table 2 lists the MEDM estimates for two lipid classes of the GL category: DAGs (n = 24) and TAGs (n = 59). Table 3 lists the estimates for the numerous lipids of several classes in the GP category, including LPCs (n = 25), PEs (n = 8), PCs (n = 63), PEs (n = 35), PEs (n = 15), and PSs (n = 1). Table 4 lists the estimates for three classes in the SP category, including CERs (n = 15), HexCers (n = 5), and SMs (n = 38). Table 5 lists the estimates for the ST category, including CEs (n = 19), BAs (n = 14), and FC. These ST lipids represent about 59% of the total lipid concentration of SRM 1950 (See Fig. 1B).

Additional consensus location values for those lipids with only three to four laboratories reporting (n = 192) are listed in supplemental Table S6 to expand the lipidome coverage for SRM 1950. These “tentative” values are calculated using the DSL estimator, which is more reliable than the MEDM with small numbers of normally distributed data (62). For inclusion as a tentative location, we set the criteria at having a DSL-based COD ≤ 20% and the percent difference between the DSL and MEDM estimates ≤ 20%.

There were 62 lipids that fit this criterion (supplemental values were calculated for those lipids measured by at least five laboratories and had COD values < 40%.

| Lipid       | Number of Laboratories | Units | Consensus Location | Standard Uncertainty | COD (%) |
|-------------|------------------------|-------|--------------------|----------------------|---------|
| FFA 16:0    | 5                      | nmol/ml | 43                  | 13                   | 31      |
| FFA 18:3    | 6                      | nmol/ml | 2.9                 | 0.62                 | 21      |
| FFA 20:4    | 7                      | nmol/ml | 4.7                 | 1.5                  | 31      |
| FFA 20:5    | 7                      | nmol/ml | 0.42                | 0.056                | 13      |
| FFA 22:6    | 8                      | nmol/ml | 1.5                 | 0.17                 | 11      |
| 5-HETE      | 5                      | pmol/ml | 10                  | 1.3                  | 13      |
| 12-HETE     | 5                      | pmol/ml | 6.8                 | 1.5                  | 23      |
| 15-HETE     | 5                      | pmol/ml | 2.4                 | 0.64                 | 27      |

MEDM consensus estimates shown were calculated for those lipids measured by at least five laboratories and had COD values < 40%.
Interlaboratory comparison exercise for lipidomics using SRM 1950

used to evaluate different sources of variability (e.g., sample preparation, instrumental data acquisition, and analysis), determine the long-term robustness of measurement processes, and validate methods (73). SRM 1950 is a CRM produced by NIST with certified reference values for amino acids, cholesterol, vitamins, total fatty acids, and other clinical markers. While the consensus values generated for SRM 1950 in this interlaboratory study are not certified, the values are a cross-section of measurements obtained within the lipidomics community using a CRM with which researchers can assess measurement methodology (e.g., quantitation performance). The calculated consensus locations provide the lipidomics community with the opportunity to extend quality control activities beyond the typical practices performed internally using in-house materials. On a wider scale, SRM 1950 has 339 robustly measured lipids (Table S6), largely represented by eicosanoids (n = 20) and TAGs (n = 7). One lipid with a tentative value was total cholesterol, which has a NIST-certified concentration of 3,917 ± 85 nmol/ml reported on the SRM 1950 COA. The DSL estimate for total cholesterol, as calculated using the interlaboratory submissions, was 3,980 ± 24 nmol/ml, which was within the uncertainty of the certified reference value note on the COA.

Usefulness of final consensus values

CRMs are widely employed to assess measurement methodologies. For example, a laboratory can have confidence that the process or method employed provided a quality measurement if their measured value agrees with the certified value within the combined uncertainties of the measured and certified values. Moreover, CRMs can also be used to evaluate different sources of variability (e.g., sample preparation, instrumental data acquisition, and analysis), determine the long-term robustness of measurement processes, and validate methods (73). SRM 1950 is a CRM produced by NIST with certified reference values for amino acids, cholesterol, vitamins, total fatty acids, and other clinical markers. While the consensus values generated for SRM 1950 in this interlaboratory study are not certified, the values are a cross-section of measurements obtained within the lipidomics community using a CRM with which researchers can assess measurement methodology (e.g., quantitation performance). The calculated consensus locations provide the lipidomics community with the opportunity to extend quality control activities beyond the typical practices performed internally using in-house materials. On a wider scale, SRM 1950 has 339 robustly measured lipids.

Table S6), largely represented by eicosanoids (n = 20) and TAGs (n = 7). One lipid with a tentative value was total cholesterol, which has a NIST-certified concentration of 3,917 ± 85 nmol/ml reported on the SRM 1950 COA. The DSL estimate for total cholesterol, as calculated using the interlaboratory submissions, was 3,980 ± 24 nmol/ml, which was within the uncertainty of the certified reference value note on the COA.

Usefulness of final consensus values

CRMs are widely employed to assess measurement methodologies. For example, a laboratory can have confidence that the process or method employed provided a quality measurement if their measured value agrees with the certified value within the combined uncertainties of the measured and certified values. Moreover, CRMs can also be used to evaluate different sources of variability (e.g., sample preparation, instrumental data acquisition, and analysis), determine the long-term robustness of measurement processes, and validate methods (73). SRM 1950 is a CRM produced by NIST with certified reference values for amino acids, cholesterol, vitamins, total fatty acids, and other clinical markers. While the consensus values generated for SRM 1950 in this interlaboratory study are not certified, the values are a cross-section of measurements obtained within the lipidomics community using a CRM with which researchers can assess measurement methodology (e.g., quantitation performance). The calculated consensus locations provide the lipidomics community with the opportunity to extend quality control activities beyond the typical practices performed internally using in-house materials. On a wider scale, SRM 1950 has 339 robustly measured lipids.
| Lipid        | Number of Laboratories | Units     | Consensus Location | Standard Uncertainty | COD (%) |
|--------------|-----------------------|-----------|--------------------|----------------------|---------|
| LPC 14:0     | 16                    | nmol/ml   | 1.0                | 0.20                 | 19      |
| LPC 15:0     | 9                     | nmol/ml   | 0.52               | 0.11                 | 22      |
| LPC 16:0     | 20                    | nmol/ml   | 73                 | 11                   | 15      |
| LPC O-16:0   | 10                    | nmol/ml   | 0.55               | 0.16                 | 29      |
| LPC P-16:0   | 8                     | nmol/ml   | 0.46               | 0.13                 | 27      |
| LPC 16:1     | 19                    | nmol/ml   | 2.4                | 0.35                 | 15      |
| LPC 17:0     | 6                     | nmol/ml   | 1.4                | 0.24                 | 18      |
| LPC 17:1     | 6                     | nmol/ml   | 0.25               | 0.071                | 29      |
| LPC 18:0     | 20                    | nmol/ml   | 27                 | 3.3                  | 12      |
| LPC O-18:0   | 6                     | nmol/ml   | 0.16               | 0.058                | 56      |
| LPC 18:1     | 19                    | nmol/ml   | 18                 | 2.5                  | 13      |
| LPC 18:2     | 19                    | nmol/ml   | 22                 | 2.0                  | 15      |
| LPC 18:3     | 18                    | nmol/ml   | 0.44               | 0.13                 | 30      |
| LPC 20:0     | 7                     | nmol/ml   | 0.10               | 0.034                | 34      |
| LPC 20:1     | 13                    | nmol/ml   | 0.19               | 0.024                | 12      |
| LPC 20:2     | 9                     | nmol/ml   | 0.23               | 0.044                | 19      |
| LPC 20:3     | 18                    | nmol/ml   | 1.8                | 0.26                 | 15      |
| LPC 20:4     | 20                    | nmol/ml   | 6.0                | 0.60                 | 10      |
| LPC 20:5     | 15                    | nmol/ml   | 0.33               | 0.092                | 28      |
| LPC 22:0     | 5                     | nmol/ml   | 0.025              | 0.0017               | 7       |
| LPC 22:1     | 5                     | nmol/ml   | 0.013              | 0.0046               | 36      |
| LPC 22:4     | 8                     | nmol/ml   | 0.12               | 0.041                | 33      |
| LPC 22:5     | 12                    | nmol/ml   | 0.43               | 0.13                 | 50      |
| LPC 22:6     | 17                    | nmol/ml   | 0.77               | 0.14                 | 18      |
| LPE 16:0     | 14                    | nmol/ml   | 0.046              | 0.015                | 58      |
| LPE 18:0     | 15                    | nmol/ml   | 1.6                | 0.55                 | 34      |
| LPE 18:1     | 14                    | nmol/ml   | 1.4                | 0.47                 | 35      |
| LPE 18:2     | 16                    | nmol/ml   | 1.9                | 0.56                 | 30      |
| LPE 20:4     | 12                    | nmol/ml   | 0.52               | 0.18                 | 34      |
| PC 30:0      | 11                    | nmol/ml   | 1.6                | 0.32                 | 20      |
| PC O-30:0/29:0| 7                   | nmol/ml   | 0.072              | 0.026                | 36      |
| PC O-30:1/P-30:0| 7           | nmol/ml   | 0.047              | 0.0096               | 20      |
| PC 32:0      | 18                    | nmol/ml   | 7.2                | 1.0                  | 14      |
| PC O-32:0/31:0| 11                  | nmol/ml   | 1.5                | 0.41                 | 28      |
| PC 32:1      | 18                    | nmol/ml   | 13                 | 1.9                  | 15      |
| PC O-32:1/P-32:0/31:1| 11    | nmol/ml   | 1.6                | 0.24                 | 14      |
| PC O-32:2/P-32:1/31:2| 8     | nmol/ml   | 0.34               | 0.093                | 28      |
| PC 32:3      | 8                     | nmol/ml   | 0.42               | 0.14                 | 34      |
| PC P-33:1/32:2| 16                  | nmol/ml   | 2.6                | 0.37                 | 14      |
| PC 34:0      | 12                    | nmol/ml   | 2.1                | 0.57                 | 18      |
| PC O-34:0/33:0| 10                  | nmol/ml   | 0.76               | 0.17                 | 22      |
| PC 34:1      | 19                    | nmol/ml   | 1.20               | 0.21                 | 17      |
| PC O-34:1/P-34:0/33:1| 17    | nmol/ml   | 4.9                | 0.86                 | 17      |
| PC O-34:2/P-34:1/33:2| 17   | nmol/ml   | 5.2                | 1.3                  | 25      |
| PC O-34:3/P-34:2/33:3| 12    | nmol/ml   | 4.7                | 0.88                 | 19      |
| PC P-35:1/34:2| 18                  | nmol/ml   | 240                | 47                   | 19      |
| PC 35:2/34:3| 18                    | nmol/ml   | 12                 | 1.7                  | 14      |
| PC O-35:4/34:4| 9                   | nmol/ml   | 1.0                | 0.7                  | 24      |
| PC 34:5      | 5                     | nmol/ml   | 0.034              | 0.0045               | 13      |
| PC 36:1      | 17                    | nmol/ml   | 26                 | 4.6                  | 17      |
| PC O-36:1/P-36:0/35:1| 16    | nmol/ml   | 3.5                | 0.99                 | 28      |
| PC 36:2      | 18                    | nmol/ml   | 140                | 25                   | 17      |
| PC O-36:2/P-36:1/35:2| 17    | nmol/ml   | 7.4                | 1.7                  | 22      |
| PC 36:3      | 17                    | nmol/ml   | 100                | 14                   | 14      |
| PC O-36:3/P-36:2/35:3| 12    | nmol/ml   | 3.7                | 0.82                 | 22      |
| PC 36:4      | 19                    | nmol/ml   | 150                | 28                   | 19      |
| PC O-36:4/P-36:3/35:4| 17    | nmol/ml   | 12                 | 1.4                  | 12      |
| PC 36:5      | 16                    | nmol/ml   | 11                 | 1.8                  | 17      |
| PC O-36:5/P-36:4/35:5| 11    | nmol/ml   | 6.9                | 1.6                  | 23      |
| PC P-36:5/35:6| 5                   | nmol/ml   | 0.30               | 0.094                | 31      |
| PC 36:6      | 8                     | nmol/ml   | 0.28               | 0.088                | 32      |
| PC 38:2      | 15                    | nmol/ml   | 2.3                | 0.20                 | 9       |
| PC O-38:2/37:2| 6                   | nmol/ml   | 0.98               | 0.32                 | 32      |
| PC 38:3      | 14                    | nmol/ml   | 26                 | 5.2                  | 20      |
| PC O-38:3/P-38:2/37:3| 14    | nmol/ml   | 1.5                | 0.31                 | 54      |
| PC 38:4      | 18                    | nmol/ml   | 84                 | 14                   | 17      |
| PC O-38:4/P-38:3/37:4| 12    | nmol/ml   | 7.4                | 2.0                  | 27      |
| PC 38:5      | 18                    | nmol/ml   | 42                 | 7.9                  | 19      |
| PC O-38:5/P-38:4/37:5| 16    | nmol/ml   | 11                 | 1.6                  | 14      |
| PC 38:6      | 18                    | nmol/ml   | 41                 | 4.4                  | 11      |
| PC O-38:6/P-38:5/37:6| 12    | nmol/ml   | 3.6                | 1.0                  | 29      |
Interlaboratory comparison exercise for lipidomics using SRM 1950

Comparison of consensus locations to LIPID MAPS consortium concentrations

The calculated consensus values were compared with the lipid concentrations noted in a report by Quehenberger et al. (14) where lipids were investigated in SRM 1950 by several members of the LIPID MAPS consortium using targeted (class-specific) methods. It is important to note that this interlaboratory study was unique in that the LIPID MAPS study only employed a single expert laboratory for each lipid class using predominantly triple quadrupole

| Lipid          | Number of Laboratories | Units   | Consensus Location | Standard Uncertainty | COD (%) |
|----------------|------------------------|---------|--------------------|----------------------|---------|
| PC P-38:6/36:0 | 10                     | nmol/ml | 1.2                | 0.39                 | 33      |
| PC 40:4        | 18                     | nmol/ml | 2.9                | 0.37                 | 13      |
| PC O-40:2/P-40:1 | 5                   | nmol/ml | 0.069              | 0.021                | 30      |
| PC O-40:4/P-40:5/39:4 | 8              | nmol/ml | 0.95               | 0.38                 | 40      |
| PC 40:5        | 18                     | nmol/ml | 6.7                | 1.1                  | 16      |
| PC O-40:5/P-40:4/39:5 | 12             | nmol/ml | 1.7                | 0.45                 | 27      |
| PC 40:6        | 17                     | nmol/ml | 14                 | 2.6                  | 19      |
| PC 40:7        | 16                     | nmol/ml | 3.5                | 0.76                 | 21      |
| PC O-40:7/P-40:6/39:7 | 9               | nmol/ml | 1.1                | 0.23                 | 20      |
| PC 40:8        | 14                     | nmol/ml | 0.73               | 0.20                 | 28      |
| PC O-42:5/P-42:4 | 7                  | nmol/ml | 0.79               | 0.12                 | 15      |
| PE 32:1        | 14                     | nmol/ml | 0.34               | 0.12                 | 36      |
| PE 34:1        | 14                     | nmol/ml | 1.2                | 0.17                 | 14      |
| PE O-34:2/P-34:1 | 11               | nmol/ml | 0.78               | 0.17                 | 22      |
| PE O-34:3/P-34:2 | 11               | nmol/ml | 1.5                | 0.41                 | 27      |
| PE 36:0        | 11                     | nmol/ml | 0.28               | 0.10                 | 36      |
| PE 36:1        | 14                     | nmol/ml | 1.3                | 0.26                 | 20      |
| PE 36:2        | 16                     | nmol/ml | 6.7                | 0.79                 | 12      |
| PE O-36:2/P-36:1/35:2 | 12             | nmol/ml | 0.93               | 0.22                 | 23      |
| PE 36:3        | 16                     | nmol/ml | 2.4                | 0.38                 | 16      |
| PE O-36:3/P-36:2/35:3 | 15            | nmol/ml | 3.2                | 0.76                 | 24      |
| PE 36:4        | 16                     | nmol/ml | 3.1                | 0.39                 | 13      |
| PE O-36:4/P-36:3 | 14              | nmol/ml | 1.6                | 0.29                 | 18      |
| PE O-36:5/P-36:4 | 15              | nmol/ml | 4.9                | 1.9                  | 38      |
| PE 38:3        | 14                     | nmol/ml | 0.95               | 0.20                 | 21      |
| PE 38:4        | 16                     | nmol/ml | 8.1                | 1.2                  | 15      |
| PE O-38:4/P-38:3/37:4 | 9               | nmol/ml | 0.94               | 0.18                 | 19      |
| PE 38:5        | 12                     | nmol/ml | 2.7                | 0.47                 | 17      |
| PE O-38:5/P-38:4 | 17              | nmol/ml | 5.8                | 1.9                  | 33      |
| PE 38:6        | 15                     | nmol/ml | 3.2                | 0.59                 | 19      |
| PE O-38:6/P-38:5 | 16              | nmol/ml | 4.9                | 1.2                  | 25      |
| PE O-38:7/P-38:6 | 8               | nmol/ml | 3.5                | 0.98                 | 28      |
| PE 40:4        | 10                     | nmol/ml | 0.26               | 0.082                | 31      |
| PE 40:5        | 12                     | nmol/ml | 0.73               | 0.23                 | 31      |
| PE O-40:5/P-40:4/39:5 | 12            | nmol/ml | 0.73               | 0.13                 | 17      |
| PE 40:6        | 14                     | nmol/ml | 1.8                | 0.36                 | 20      |
| PE O-40:6/P-40:5/39:6 | 14            | nmol/ml | 1.3                | 0.31                 | 23      |
| PE 40:7        | 11                     | nmol/ml | 0.77               | 0.26                 | 33      |
| PE O-40:7/P-40:6/39:7 | 14            | nmol/ml | 2.5                | 0.72                 | 29      |
| PI 32:1        | 10                     | nmol/ml | 0.56               | 0.11                 | 19      |
| PI 34:1        | 14                     | nmol/ml | 2.4                | 0.42                 | 17      |
| PI 34:2        | 14                     | nmol/ml | 2.8                | 0.38                 | 14      |
| PI 36:1        | 13                     | nmol/ml | 2.1                | 0.59                 | 28      |
| PI 36:2        | 15                     | nmol/ml | 7.7                | 0.93                 | 12      |
| PI 36:3        | 14                     | nmol/ml | 2.2                | 0.29                 | 14      |
| PI 36:4        | 14                     | nmol/ml | 3.0                | 0.48                 | 16      |
| PI 38:3        | 14                     | nmol/ml | 3.4                | 0.54                 | 16      |
| PI 38:4        | 17                     | nmol/ml | 1.9                | 2.2                  | 11      |
| PI 38:5        | 15                     | nmol/ml | 2.5                | 0.44                 | 18      |
| PI 38:6        | 10                     | nmol/ml | 0.32               | 0.031                | 10      |
| PI 40:4        | 7                      | nmol/ml | 0.30               | 0.042                | 14      |
| PI 40:6        | 12                     | nmol/ml | 0.84               | 0.16                 | 19      |
| PG 36:2        | 6                      | nmol/ml | 0.67               | 0.24                 | 36      |
technology. Therefore, the LIPID MAPS study did not provide information on the state of lipid measurements across the community at large or include methods using both targeted and untargeted workflows with the latest instrumentation. In total, the LIPID MAPS study reported 588 lipids in SRM 1950 from several lipid classes, while the interlaboratory exercise reported 1,527 individual lipid species. A comparison of the reported LIPID MAPS species to those reported in the interlaboratory exercise (by five or more laboratories) resulted in 226 overlapping lipid species. A comparison of these overlapping species, organized by lipid class, is shown in supplemental Tables S7–S16. The individual MEDM and LIPID MAPS study values were also summed by lipid class and the results (derived values in supplemental Table S17) were compared in Fig. 3A (high concentration lipids) and Fig. 3B (low concentration lipids). The sum of the 226 lipids in common from the LIPID MAPS study (8,438 ± 106 nmol/ml) was significantly higher than that of the same lipid species determined in this exercise (6,218 ± 475 nmol/ml). As shown in Fig. 3A, B, this difference was driven mostly by PC, PE, and TAG species. The main contributors to the difference between the two studies were phospholipids and, to a lesser extent, nonpolar lipids. This coincided with a large percent change in the interlaboratory consensus estimates relative to the LIPID MAPS measurements, with percent changes: LPCs (+48%), LPEs (−80%), PCs (−56%), PEs (−83%), PIs (+58%), and TAGs (−54%). In addition to methodological differences, reporting at the sum composition level might contribute to some of these differences, as the isomer lipids contributing to the sums may not be the same. Overall, the total lipid content for common lipids showed that the LIPID MAPS sum was 30% larger than the summed composition of common lipids that were determined in this exercise, signifying a difference in measurement effects between studies, an aspect that will be addressed with future efforts.

### Future of lipidomic quantitation

To date, no clear community-wide consensus exists for the best approach to quantify lipids. Quantitation in lipidomics

| Lipid          | Number of Laboratories | Units     | Consensus Location | Standard Uncertainty | COD (%) |
|----------------|------------------------|-----------|--------------------|----------------------|---------|
| HexCer d34:1   | 6                      | nmol/ml   | 0.86               | 0.21                 | 25      |
| HexCer d36:1   | 5                      | nmol/ml   | 0.13               | 0.043                | 34      |
| HexCer d40:1   | 5                      | nmol/ml   | 2.4                | 0.68                 | 28      |
| HexCer d42:1   | 6                      | nmol/ml   | 2.7                | 0.73                 | 27      |
| CER d34:1      | 17                     | nmol/ml   | 0.28               | 0.044                | 16      |
| CER d36:1      | 14                     | nmol/ml   | 0.12               | 0.021                | 17      |
| CER d38:1      | 16                     | nmol/ml   | 0.11               | 0.021                | 20      |
| CER d40:1      | 18                     | nmol/ml   | 0.65               | 0.12                 | 18      |
| CER d40:2      | 6                      | nmol/ml   | 0.15               | 0.021                | 14      |
| CER d41:1      | 7                      | nmol/ml   | 0.67               | 0.27                 | 40      |
| CER d42:1      | 19                     | nmol/ml   | 1.9                | 0.47                 | 24      |
| CER d43:2      | 19                     | nmol/ml   | 0.82               | 0.10                 | 12      |
| SM d31:1       | 5                      | nmol/ml   | 0.19               | 0.049                | 25      |
| SM d32:1       | 14                     | nmol/ml   | 0.43               | 0.29                 | 23      |
| SM d32:2       | 10                     | nmol/ml   | 0.66               | 0.24                 | 36      |
| SM d33:1       | 14                     | nmol/ml   | 2.7                | 0.12                 | 14      |
| SM d34:0       | 14                     | nmol/ml   | 5.8                | 1.3                  | 22      |
| SM d34:1       | 21                     | nmol/ml   | 100                | 15                   | 15      |
| SM d34:2       | 17                     | nmol/ml   | 16                 | 2.2                  | 14      |
| SM d35:1       | 9                      | nmol/ml   | 2.5                | 0.58                 | 23      |
| SM d35:2       | 6                      | nmol/ml   | 0.52               | 0.21                 | 39      |
| SM d36:0       | 11                     | nmol/ml   | 2.0                | 0.49                 | 24      |
| SM d36:1       | 22                     | nmol/ml   | 20                 | 3.7                  | 18      |
| SM d36:2       | 22                     | nmol/ml   | 9.6                | 1.5                  | 16      |
| SM d36:3       | 15                     | nmol/ml   | 1.3                | 0.41                 | 31      |
| SM d37:1       | 11                     | nmol/ml   | 1.0                | 0.23                 | 23      |
| SM d38:1       | 17                     | nmol/ml   | 11                 | 3.1                  | 27      |
| SM d38:2       | 17                     | nmol/ml   | 2.7                | 1.3                  | 25      |
| SM d38:3       | 8                      | nmol/ml   | 0.61               | 0.24                 | 39      |
| SM d39:1       | 14                     | nmol/ml   | 3.1                | 1.0                  | 29      |
| SM d39:2       | 9                      | nmol/ml   | 0.61               | 0.16                 | 26      |
| SM d40:1       | 17                     | nmol/ml   | 20                 | 5.1                  | 25      |
| SM d40:2       | 15                     | nmol/ml   | 12                 | 2.8                  | 24      |
| SM d40:3       | 8                      | nmol/ml   | 2.2                | 0.79                 | 37      |
| SM d41:1       | 14                     | nmol/ml   | 7.7                | 2.1                  | 27      |
| SM d41:2       | 14                     | nmol/ml   | 5.8                | 1.4                  | 24      |
| SM d41:3       | 7                      | nmol/ml   | 0.77               | 0.30                 | 30      |
| SM d42:1       | 21                     | nmol/ml   | 20                 | 5.4                  | 28      |
| SM d42:2       | 18                     | nmol/ml   | 4.4                | 1.1                  | 25      |
| SM d42:3       | 12                     | nmol/ml   | 17                 | 4.7                  | 27      |
| SM d43:2       | 10                     | nmol/ml   | 1.0                | 0.29                 | 29      |
| SM d44:2       | 9                      | nmol/ml   | 0.40               | 0.15                 | 32      |

**MEDM consensus estimates shown were calculated for those lipids measured by at least five laboratories and had COD values ≤40%.**
is a polarizing subject within the community, with both methodological and philosophical differences to consider. The community has limited agreement on the definition of current quantitation approaches (absolute, semi-, and relative) and determination of the essential guidelines to perform each approach. Furthermore, the discussion of quantitation becomes more convoluted when assessing strategies for both targeted and global profiling approaches because neither has been explicitly studied. There is a quantitation tradeoff between these two approaches. Generally, targeted approaches employ calibration curves and appropriate standards, which improve quantitation, while global approaches typically provide more lipid identifications in a single analysis. Even in targeted studies for lipidomics, appropriate standards are often not available and single point calibration is commonly used. The lipidomics community is implementing relative quantitation experiments to increase accuracy in untargeted studies, with a focus on monitoring lipid species changes between sample groups rather than determining the exact concentration of lipids (74–76). Laboratories generally employ semi-quantitative approaches to provide concentrations for lipid species; however, several assumptions are generally made using this approach (32, 76–78).

One major impediment to uniform quantitation within the community is the lack of suitable internal standards. To date, several different types of internal standards have been employed (odd-chained, deuterated, or $^{13}$C-labeled), but each has limitations. Ideally, multiple internal standards should be employed for all types and classes of lipids to improve quantitation. However, the availability of lipids that can serve as internal standards is limited. In this study, the specific internal standards utilized largely influenced the reported final lipid concentration. For example, if a laboratory quantified a lipid class with an internal standard

| Lipid | Number of Laboratories | Units | Consensus Location | Standard Uncertainty | COD (%) |
|-------|------------------------|-------|--------------------|----------------------|---------|
| CE 14:0 | 7 | nmol/ml | 16 | 6.0 | 37 |
| CE 15:0 | 6 | nmol/ml | 5.3 | 1.8 | 34 |
| CE 16:0 | 13 | nmol/ml | 210 | 58 | 28 |
| CE 16:1 | 11 | nmol/ml | 100 | 27 | 27 |
| CE 16:2 | 5 | nmol/ml | 1.9 | 0.46 | 25 |
| CE 17:1 | 9 | nmol/ml | 8.2 | 1.0 | 13 |
| CE 18:0 | 7 | nmol/ml | 15 | 3.7 | 25 |
| CE 18:1 | 14 | nmol/ml | 450 | 110 | 25 |
| CE 18:2 | 14 | nmol/ml | 1,700 | 430 | 26 |
| CE 18:3 | 13 | nmol/ml | 84 | 24 | 28 |
| CE 20:3 | 13 | nmol/ml | 35 | 12 | 35 |
| CE 20:4 | 14 | nmol/ml | 350 | 58 | 17 |
| CE 20:5 | 12 | nmol/ml | 38 | 8.6 | 23 |
| CE 22:5 | 6 | nmol/ml | 4.1 | 1.6 | 39 |
| CE 22:6 | 11 | nmol/ml | 37 | 9.5 | 26 |
| Cholesterol | 8 | nmol/ml | 770 | 110 | 14 |
| CDCA | 7 | nmol/ml | 0.30 | 0.11 | 38 |
| CA | 9 | nmol/ml | 0.12 | 0.034 | 28 |
| DCA | 9 | nmol/ml | 0.35 | 0.083 | 24 |
| GCDCDA | 8 | nmol/ml | 1.1 | 0.18 | 17 |
| GDCA | 7 | nmol/ml | 0.43 | 0.069 | 16 |
| GLCA | 6 | nmol/ml | 0.025 | 0.0018 | 7 |
| GDCA | 6 | nmol/ml | 0.15 | 0.024 | 16 |
| GCA | 6 | nmol/ml | 0.24 | 0.069 | 29 |
| LCA | 26 | nmol/ml | 0.014 | 0.0036 | 26 |
| TCDDCA | 9 | nmol/ml | 0.084 | 0.0050 | 6 |
| TCA | 9 | nmol/ml | 0.026 | 0.0056 | 22 |
| TDCA | 8 | nmol/ml | 0.040 | 0.0064 | 16 |
| TLCA | 5 | nmol/ml | 0.0027 | 0.00069 | 26 |
| UDCA | 8 | nmol/ml | 0.11 | 0.024 | 22 |

CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; GCA, glycocholic acid; GCDCDA, glycochenodeoxycholic acid; GDCA, glycodeoxycholic acid; GLCA, glycolithocholic acid; GDCA, glycochenodeoxycholic acid; DCA, deoxycholic acid; GCA, glycocholic acid; GCDCDA, glycodeoxycholic acid; DCA, deoxycholic acid; GCA, glycocholic acid; GCDCDA, glycodeoxycholic acid; LCA, lithocholic acid; TCA, taurocholic acid; TCDCA, taurochenodeoxycholic acid; TCA, taurocholic acid; TCDCA, taurochenodeoxycholic acid; TCA, taurocholic acid; TCA, taurocholic acid. MEDM consensus estimates shown were calculated for those lipids measured by at least five laboratories and had COD values <40%.

**Fig. 2.** COD (in percent) for the MEDM lipids ($n \geq 5$ laboratories reporting) organized by lipid class. Each point on the figure represents a single sum lipid composition. The COD was calculated by dividing the standard uncertainty by the final MEDM. CODs not shown in the figure are FC, eicosanoids, PGs, and PSSs.
from a different class, often the concentration values were quite different from those obtained from laboratories using standards from the appropriate lipid class. We found that several odd-chain lipids, often used by laboratories as exogenous internal standards, were reported as endogenous lipids by participating laboratories in this exercise (e.g., CE 17:0, n = 6; LPC 17:0, n = 6; SM d35:1, n = 9; and TAG 51:3, n = 5; n indicates number of incidences).

Comparing the consensus values from this exercise (using a variety of quantitation MS platforms: triple quadrupole, quadrupole time-of-flight, and Orbitrap) to the concentration values obtained using the targeted triple quadrupole platforms, we found that the targeted approaches generally had significantly higher calculated concentration values. Future studies will further explore the contribution of analytical platforms and lipidomics workflows to the final concentration calculated using the interlaboratory data. As the community begins to develop and establish guidelines for quality assurance and quality control, discussions need to include acceptable practices for quantitation across the varying platforms present within the lipidomics community.

CONCLUSIONS

The purpose of this lipidomic interlaboratory comparison exercise was to identify the metrological questions and/or gaps that exist in current lipidomic measurement. To determine the principal areas of need, the interlaboratory exercise was initiated using a commercially available CRM, SRM 1950. This interlaboratory study provides an initial outlook into the variance associated with current lipid methodologies. The robustly measured SRM 1950 consensus estimates can be used for community-wide quality control and quality assessment. These values were compared with those previously reported by LIPID MAPS, with significant discrepancies for specific lipid classes between both studies, and thus require further attention to understand the reasons behind this difference. From a community perspective, the exercise also provided valuable insight into the potential strengths and weaknesses of current lipidomic measurement. Future efforts resulting from this interlaboratory study will focus on making the data available to the community and examining the influence that the laboratory-provided methodology had on the resultant trends in the collective data. We currently intend to provide a supplemental survey to direct future measurement efforts regarding lipidomic measurement.

The authors would like to thank Kayla Carter (US Center for Disease Control and Prevention), Debra Ellisor (NIST), John Kucklick (NIST), Amanda Moors (NIST), Katrice Lippa (NIST), Dave Duewer (NIST), and William Blaner (Columbia University II CTR Core Laboratory) for their contributions.

REFERENCES

1. Burr, G. O., and M. M. Burr. 1929. A new deficiency disease produced by the rigid exclusion of fat from the diet. *J. Biol. Chem.* **82**: 345–367.
2. Burr, G. O., and M. M. Burr. 1930. On the nature and role of the fatty acids essential in nutrition. *J. Biol. Chem.* **86**: 587–621.
3. Steinberg, D. 2004. An interpretive history of the cholesterol controversy: part I. *J. Lipid Res.* **45**: 1583–1593.
4. Steinberg, D. 2005. An interpretive history of the cholesterol controversy: part II: the early evidence linking hypercholesterolemia to coronary disease in humans. *J. Lipid Res.* **46**: 179–190.
5. Wilson, P. W. 2013. Lipids and vascular disease: a Framingham perspective. *Glob. Heart.* **8**: 25–33.
6. Blanksy, S. J., and T. W. Mitchell. 2010. Advances in mass spectrometry for lipidomics. *Annu. Rev. Anal. Chem. (Palo Alto Calif).* **3**: 433–465.
7. Harkewicz, R., and E. A. Dennis. 2011. Applications of mass spectrometry to lipids and membranes. *Annu. Rev. Biochem.* **80**: 301–325.
8. Queneberger, O., and E. A. Dennis. 2011. The human plasma lipidome. *N. Engl. J. Med.* **365**: 1812–1823.
9. Murphy, S. A., and A. Nicolau. 2013. Lipidomics applications in health, disease and nutrition research. Mol. Nutr. Food Res. 57: 1336–1346.

10. Hu, C., R. van der Heijden, M. Wang, J. van der Greef, T. Hankemeier, and G. Xu. 2009. Analytical strategies in lipidomics and application in disease biomarker discovery. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 877: 2836–2846.

11. Yang, L., M. Li, Y. Shan, S. Shen, Y. Bai, and H. Liu. 2016. Recent advances in lipidsomics for disease research. J. Sep. Sci. 39: 38–50.

12. Dennis, E. A., R. A. Deems, C. K. Glass, A. H. Merrill, R. C. Murphy, R. H. Raetz, W. Shaw, S. Subramaniam, D. W. Russell, and S. Le Bizec. 2013. Versatile lipid profiling by liquid chromatography–high resolution mass spectrometry using all ion fragmentation and polarity switching. Preliminary application for serum samples phenotyping related to canine mammary cancer. Anal. Chem. Acta. 796: 75–83.

13. Dennis, E. A. 2009. Lipidomics joins the omics evolution. Proc. Natl. Acad. Sci. USA. 106: 2089–2090.

14. Quenchnerber, O., A. M. Armando, A. H. Brown, S. B. Milne, D. S. Myers, A. H. Merrill, S. Bandyopadhyay, K. N. Jones, S. Kelly, and R. L. Shaner. 2010. Lipidomics reveals a remarkable diversity of lipids in human plasma. J. Lipid Res. 51: 3299–3305.

15. Caja, T., and O. Fieth. 2014. Comprehensive analysis of lipids in biological systems by liquid chromatography-mass spectrometry. Trends Analys. Chem. 61: 192–206.

16. Gallart-Ayala, H., F. Courant, S. Severe, J-P. Antignac, F. Morio, J. Alejandro, and S. Le Bizec. 2013. Versatile lipid profiling by liquid chromatography–high resolution mass spectrometry using all ion fragmentation and polarity switching. Preliminary application for serum samples phenotyping related to canine mammary cancer. Anal. Chem. Acta. 796: 75–83.

17. Yamada, T., T. Uchikata, S. Sakamoto, Y. Yokoi, E. Fukusaki, and T. Bamba. 2013. Development of a lipid profiling system using reverse-phase liquid chromatography coupled to high-resolution mass spectrometry with rapid polarity switching and an automated lipid identification software. J. Chromatogr. A. 1292: 211–218.

18. Caja, T., and O. Fieth. 2016. Toward merging untargeted and targeted methods in mass spectrometry-based metabolomics and lipidomics. Anal. Chem. 88: 524–545.

19. Hyöryläinen, T., and M. Oresič. 2016. Bioanalytical techniques in nontargeted clinical lipidomics. Bioanalysis. 8: 351–364.

20. Gross, R. W., and X. Han. 2011. Lipidomics at the interface of structure and function in systems biology. Chem. Biol. 18: 284–291.

21. Shevchenko, A., and K. Simons. 2010. Lipidomics: coming to grips with lipid diversity. Nat. Rev. Mol. Cell Biol. 11: 593–598.

22. Ekkro, K., M. Jänis, K. Tarasov, R. Hurme, and R. Laaksonen. 2010. Lipidomics: a tool for studies of atherosclerosis. Curr. Atheroscler. Rep. 12: 273–281.

23. Auffray, C., T. Caulfield, J. L. Griffin, M. J. Khoury, J. R. Lupski, and M. Schwab. 2016. From genomic medicine to precision medicine: highlights of 2015. Genome Med. 8: 12.

24. Abadie, A. 2010. Mass spectrometry: an emerging but powerful tool for precision medicine. Cold Spring Harb. Mol. Case Stud. 1: a000588.

25. Looma, R., O. Quenchnerber, A. Armando, and E. A. Dennis. 2015. Polyunsaturated fatty acid metabolites as novel lipidomic biomarkers for noninvasive diagnosis of nonalcoholic steatohepatitis. J. Lipid Res. 56: 185–192.

26. Gorden, D. L., D. S. Myers, P. T. Ivanova, E. Fayh, M. R. Maurya, S. Gupta, J. Min, N. J. Sporn, J. G. McDonald, S. L. Kelly, et al. 2015. Biomarkers of NAFLD progression: a lipidomics approach to an epi- demic. J. Lipid Res. 56: 722–756.

27. Teo, C. C., W. P. K. Chong, E. Tan, N. B. Basri, Z. J. Low, and Y. S. Ho. 2015. Advances in sample preparation and analytical techniques for lipidomics study of clinical samples. Trends Analys. Chem. 66: 1–18.

28. Vesper, H. W., G. L. Myers, and W. G. Miller. 2016. Current practices and challenges in the standardization and harmonization of clinical laboratory tests. Am. J. Clin. Nutr. 104: 9078–9128.

29. McShane, L. M., M. M. Cavenagh, T. G. Lively, D. A. Eberhard, W. L. Bigbee, P. W. Williams, J. P. Mesirov, M.Y. C. Polley, K. Y. Kim, and J. V. Tricoli. 2015. Criteria for the use of omics-based predictors in clinical trials explanation and elaboration. BMC Med. 11: 229.

30. Ekkro, K. 2012. Lipidomics perspective: from molecular lipids to validated clinical diagnostics. In Lipidomics: Technologies and Applications. K. Ekkro, editor. Wiley-VCH, Weinheim, Germany. 1–19.

31. Hyöryläinen, T., and M. Oresič. 2015. Optimizing the lipidomics workflow for clinical studies—practical considerations. Anal. Bioanal. Chem. 407: 4973–4995.
50. Koal, T., and H-P. Deigner. 2010. Challenges in mass spectrometry based targeted metabolomics. Curr. Mol. Med. 10: 216–226.

51. Telu, K. H., X. Yan, W. E. Wallace, S. E. Stein, and Y. Simón-Manso. 2016. Analysis of human plasma metabolites across different liquid chromatography/mass spectrometry platforms: cross-platform transferable chemical signatures. Rapid Commun. Mass Spectrom. 30: 581–593.

52. Colas, R. A., M. Shinohara, J. Dalli, N. Chiang, and C. N. Serhan. 2014. Identification and signature profiles for pro-resolving and inflammatory lipid mediators in human tissue. Am. J. Physiol. Cell Physiol. 307: C89–C94.

53. National Institute of Standards and Technology. 2012. Certificate of Analysis: Standard Reference Material 1950 - Metabolites in Human Plasma. Accessed July 5, 2017, at https://www-s.nist.gov/srmors/view_cert.cfm?srm=1950.

54. Bowden, J. A., A. Heckert, C. Z. Ulmer, C. M. Jones, and R. S. Pugh. 2017. LipidPioneer: a comprehensive user-generated exact mass template for lipidomics. J. Am. Soc. Mass Spectrom. 28: 562–565.

55. Ulmer, C. Z., J. P. Koelmel, J. M. Ragland, T. J. Garrett, and J. A. Bowden. 2017. LipidPioneer: a comprehensive user-generated exact mass template for lipidomics. J. Am. Soc. Mass Spectrom. 28: 562–565.

56. Vangel, M. G., and A. L. Rukhin. 1999. Maximum likelihood analysis for heteroscedastic one-way random effects ANOVA in interlaboratory studies. Biometrics. 55: 129–136.

57. Heckert, A., and J. J. Filliben. 2016. Dataplot Reference Manual Volume I: Commands. Accessed February 2, 2017, at http://www.itl.nist.gov/div898/software/dataplot/refman1/homepage.htm.

58. CCQM. 2013. CCQM guidance note: estimation of a consensus KCRV and associated degrees of equivalence. Version 10. Accessed February 2, 2017, at https://www.bipm.org/cc/CCQM/Allowed/19/CCQM13-22_Consensus_KCRV_v10.pdf.

59. Bonett, D. G., and E. Seier. 2006. Confidence interval for a coefficient of correlation in nonnormal distributions. Biom. J. 48: 144–148.

60. Rukhin, A. L., and M. G. Vangel. 1998. Estimation of a common mean and weighted means statistics. J. Am. Stat. Assoc. 93: 303–308.

61. Vangel, M. G., and A. L. Rukhin. 1999. Maximum likelihood analysis for heteroscedastic one-way random effects ANOVA in interlaboratory studies. Biometrics. 55: 129–136.

62. DerSimonian, R., and N. Laird. 1986. Meta-analysis in clinical trials. Control. Clin. Trials. 7: 177–188.

63. Fahey, E., S. Subramaniam, H. A. Brown, C. K. Glass, A. H. Merrill, R. C. Murphy, C. R. Raetz, D. W. Russell, Y. Seyama, W. Shaw, et al. 2005. A comprehensive classification system for lipids. J. Lipid Res. 46: 839–861.

64. Fahey, E., S. Subramaniam, R. C. Murphy, M. Nishijima, C. R. Raetz, T. Shimizu, F. Spener, G. van Meer, M. J. Waklam, and E. A. Dennis. 2009. Update of the LIPID MAPS comprehensive classification system for lipids. J. Lipid Res. 50: S9–S14.

65. Sales, S., J. Graessler, S. Ciucci, R. Al-Atrib, T. Vihervaara, K. Schuhmann, D. Kauhanen, M. Syväalo, S. R. Bornstein, and M. Bickle. 2016. Gender, contraceptives and individual metabolic predisposition shape a healthy plasma lipidome. Sci. Rep. 6: 27710.

66. Qi, F., S-J. Zheng, C-S. Wú, Z-X. Jía, J-L. Zhang, and Z-P. Duan. 2014. Lipidomic profiling of plasma in patients with chronic hepatitis C infection. Anal. Bioanal. Chem. 406: 555–564.

67. Koelmel, J. P., N. M. Kroeger, E. L. Gill, C. Z. Ulmer, J. A. Bowden, R. E. Patterson, R. A. Yost, and T. J. Garrett. 2017. Expanding lipidome coverage using LC/MS/MS data-dependent acquisition with automated exclusion list generation. J. Am. Soc. Mass Spectrom. 28: 908–917.

68. Surma, M. A., R. Herzog, A. Vasilj, C. Klose, N. Christinat, D. Morin-Rivron, K. Simons, M. Masoodi, and J. L. Sampaio. 2015. An automated shotgun lipidomics platform for high throughput, comprehensive, and quantitative analysis of blood plasma intact lipids. Eur. J. Lipid Sci. Technol. 117: 1540–1549.

69. Jung, H. R., T. Sylvâne, K. M. Koistinen, K. Tarasov, D. Kauhanen, and K. Ekroos. 2011. High throughput quantitative molecular lipidomics. Biochem. Biophys. Acta. 1811: 929–934.

70. Ovčáčková, M., M. Lisa, E. Cífková, and M. Holčapek. 2016. Retention behavior of lipids in reversed-phase ultrahigh-performance liquid chromatography-electrospray ionization mass spectrometry. J. Chromatogr. A. 1450: 76–85.

71. Meikle, P. J., G. Wong, D. Tsorotes, C. K. Barlow, J. M. Weir, M. J. Christopher, G. L. MacIntosh, B. Goudey, L. Stern, and A. Kowalczyk. 2011. Plasma lipidomic analysis of stable and unstable coronary artery disease. Arterioscler. Thromb. Vasc. Biol. 31: 2723–2729.

72. Caijka, T., and O. Fiehn. 2016. Increasing lipidomic coverage by selecting optimal mobile-phase modifiers in LC-MS of blood plasma. Metabolomics. 12: 1–11.

73. Sharpless, K. E., K. A. Lippa, D. L. Duewer, and A. L. Rukhin. 2015. The ABCs of Using Standard Reference Materials in the Analysis of Foods and Dietary Supplements: A Practical Guide. NIST Special Publication: sp260-181rev1. Accessed February 2, 2017, at http://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP-260-181.pdf.

74. Breitkopf, S. B., S. J. H. Ricoult, M. Yuan, Y. Xu, D. A. Peake, B. D. Manning, and J. M. Asara. 2017. A relative quantitative positive/negative ion switching method for untargeted lipidomics via high resolution LC-MS/MS from any biological source. Metabolomics. 13: 30.

75. Gífková, E., M. Holčapek, M. Lisa, M. Ovčáčková, A. Lyčka, F. Lynen, and P. Sandra. 2012. Nontargeted quantitation of lipid classes using hydrophilic interaction liquid chromatography-electrospray ionization mass spectrometry—meets a key challenge in lipidomics. Metabolites. 1: 21–40.

76. Koivusalo, M., P. Haimi, L. Heikinheimo, R. Kostiainen, and P. Sandra. 2012. Nontargeted quantitation of lipid classes using hydrophilic interaction liquid chromatography–electrospray ionization mass spectrometry—meets a key challenge in lipidomics. Metabolites. 1: 21–40.

77. Koivusalo, M., P. Haimi, L. Heikinheimo, R. Kostiainen, and P. Sandra. 2012. Nontargeted quantitation of lipid classes using hydrophilic interaction liquid chromatography–electrospray ionization mass spectrometry—meets a key challenge in lipidomics. Metabolites. 1: 21–40.

78. Murphy, R. C., J. Fiedler, and J. Hevko. 2001. Analysis of nonvolatile lipids by mass spectrometry. Chem. Rev. 101: 479–526.