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Interlaboratory Analytical Comparison of Fatty Acid Concentrations in Serum or Plasma

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Abstract

The National Institute of Standards and Technology, in collaboration with the National Institutes of Health Office of Dietary Supplements and the Centers for Disease Control and Prevention, is conducting an accuracy-based program for improving the comparability of individual fatty acid measurements in serum and plasma. To date, two exercises of the Fatty Acid Quality Assurance Program (FAQAP) were conducted with 11 and 14 participants, respectively. The results from these two exercises indicate the need to improve the within-lab repeatability and between-lab reproducibility thus providing more confidence in the comparability of fatty acid measurements.

Keywords

fatty acids; serum; plasma; interlaboratory study; SRM 2378; SRM 1950

1. Introduction

The measurement of individual fatty acids, hydrolyzed from lipids, as opposed to total triglycerides found in serum or plasma, is becoming more important in clinical studies. Recent studies have investigated the links between increased concentrations of omega-3 fatty acids and anti-inflammatory responses such as in the severity of osteoarthritis following joint injury [1]. Correlations between the concentrations of omega-3 fatty acids in blood and breast tissue as related to breast cancer incidence [2], the associations between omega-3 fatty acids and arterial stiffness [3], and the levels of a specific omega-3 fatty acid, DHA, during

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resistance training in elderly individuals [4] have also been studied. As potential indicators of specific diseases, studies have looked at the link between serum concentrations of linoleic acid and the incidence of liver cirrhosis [5], selected fatty acids and type 2 diabetes [6], and saturated fatty acids and cardiovascular risk [7]. Mental health experts are comparing the concentrations of long chain polyunsaturated fatty acids (PUFA) in attention-deficit/hyperactivity disorder patients and their relatives [8] and investigating a potential role for long-chain PUFA in schizophrenia [9]. A recent review article [10] discusses the role of DHA in Alzheimer's disease and other brain functions. What was not addressed, however, is the accuracy and intercomparability among the data from the numerous studies of individual fatty acids in serum and plasma.

To address the accuracy of fatty acid measurements, the National Institute of Standards and Technology (NIST) in collaboration with the National Institutes of Health (NIH) has developed two Standard Reference Materials (SRMs), one plasma-based and one serum-based, characterized for the content of individual fatty acids. SRM 1950 Metabolites in Human Plasma (<http://srmd.nist.gov>) was a collaboration between NIST and NIH National Institute of Diabetes and Digestive and Kidney Disease (NIDDK); it was intended to represent "normal" human plasma [11]. SRM 2378 Fatty Acids in Frozen Human Serum was developed as a collaboration between NIST and NIH Office of Dietary Supplements (ODS) and was intended to represent three levels of fatty acids in serum, namely, a "normal" level, a level indicative of individuals taking flaxseed supplements, and a level indicative of individuals taking fish oil supplements. The value assignments of individual fatty acids in both of these SRMs (Supplemental Table 1) were based on measurements performed by NIST and the Centers for Disease Control and Prevention (CDC). Additional information on the SRMs, including descriptions of the methods used for the certification analyses, is available in the respective Certificates of Analysis, which can be found at <http://www.nist.gov/srm/>.

To begin to address the interlaboratory comparison of data, NIST, CDC, and NIH-ODS initiated an interlaboratory analytical comparison exercise for the determination of fatty acid concentrations in human serum and plasma in 2012 followed by a second exercise in 2015. This is one of three clinical quality assurance programs (QAPs) currently conducted by NIST [12] for the determination of micronutrients, vitamin D metabolites, and fatty acids in serum and plasma matrices, the Micronutrients Measurement QAP (MMQAP) [13–16], the Vitamin D QAP (VitDQAP) [17], and the Fatty Acid QAP (FAQAP), respectively. Given the similarity in the operations of these programs, NIST has consolidated the three programs into one larger program, the NIST Clinical QAP (ClinQAP). The primary goals of the ClinQAP are to support the comparability of clinical measurements through the MMQAP, VitDQAP, and the FAQAP and to monitor and support the emerging measurement needs of the clinical community. FAQAP is a performance-based program so participating laboratories are requested to use the analytical procedures that they typically use in their laboratories for these analyses and report data for those fatty acids that they typically quantify. There is no cost for participating in the FAQAP, and there is no pass/fail assessment. The results from the first two exercises of the FAQAP are presented and discussed in this paper.

2. Materials and Methods

2.1 Description of Materials

The first exercise of the FAQAP was conducted in 2012 with 11 laboratories returning data (Table 1) for the content of individual fatty acids in SRM 2378 (prior to its release as an SRM and therefore as an unknown sample) along with SRM 1950, which was distributed as the control sample with values assigned for fatty acid content. SRM 2378 consists of three serum materials collected from: (1) donors who did not take fish or flaxseed oil supplements for one month prior to collection, (2) donors who took flaxseed oil supplements for a minimum of one month prior to collection, and (3) donors who took fish oil supplements for a minimum of one month prior to collection. SRM 1950 is designed to represent “normal” human plasma. Plasma was obtained from 100 individuals (equal number of men and women) in a narrow age range (40 to 50 years) who had undergone an overnight fast prior to blood draw. The second exercise of the FAQAP was conducted in 2015 with 14 laboratories returning data (Table 1). Three unknown serum samples previously used in MMQAP studies were distributed along with one vial of each level of SRM 2378 as the control samples. For each exercise, participating laboratories were provided with four vials of each of the unknown samples and one vial of each of the control samples. Each vial contained approximately 1 mL of serum or plasma.

2.2 Instructions for Exercises

Participants were requested to perform triplicate measurements of each unknown sample using their laboratory's analytical protocols for the measurement of the concentrations of individual fatty acids currently being determined in their laboratory. In addition, they were requested to analyze three subsamples of SRM 1950 (exercise 1) or one subsample of each level of SRM 2378 (exercise 2) as the control samples. A target list of fatty acids was provided (Table 2); however, participants did not need to quantify all of these compounds and could add additional compounds when reporting data. The participants were requested to report results, using three significant figures, in units of either $\mu\text{g/g}$ or $\mu\text{mol/L}$ (μM) and to provide brief descriptions of their analytical procedures. The analytical procedures used by the laboratories are briefly summarized in Supplemental Table 2.

2.3 Data compilation

For both exercises, laboratories were assigned numerical identification codes in order of receipt of data with the exception of the NIST laboratory. In the first exercise, four laboratories reported the data in $\mu\text{g/g}$ with the remaining laboratories reporting as μM . In the second exercise, seven laboratories reported the data in $\mu\text{g/g}$, six laboratories reported the data in μM , and one laboratory reported data in mg/mL . The conversions between the units reported and the alternate units were calculated using the densities provided by each laboratory except for one laboratory in exercise 1 and five laboratories in exercise 2 did not report a density for each sample. For these laboratories, the density of each sample was assumed to be 1 g/mL for the conversion between units.

NIST, as the coordinating laboratory, calculated laboratory mean values, standard deviations, and relative standard deviations in $\mu\text{g/g}$ and in μM for the three unknown samples in each

exercise and for SRM 1950 in exercise 1. The results from all laboratories and for all the fatty acids are summarized in both tabular and chart format in the reports for each exercise [18,19] along with summaries of the methods used and notes submitted by each laboratory. In addition to the list of suggested fatty acids (Table 2), individual laboratories reported data for additional fatty acids. These data are also included in the reports that were distributed to participants at the time of the studies [18,19].

2.4 Summary of methods used in the exercises

For exercise 1, the hydrolysis and extraction methods were generally different among the laboratories except for two laboratories reporting the use of very similar procedures [18]. All laboratories used gas chromatography (GC) with either flame ionization detection (FID) or mass spectrometry (MS) following derivatization (See Supplemental Table 2). The columns used ranged from a relatively non-polar (5 % phenyl methylpolysiloxane phase) to relatively polar (cyanopropyl phase). For calibration, five laboratories used linear regression, one laboratory used quadratic regression, one laboratory reported only a weighting factor, one laboratory did a one-point calibration, and one laboratory used a mix of linear and quadratic regression tailored by analyte. The remaining two laboratories did not report calibration information.

For exercise 2, the hydrolysis and extraction methods reported by two sets of three laboratories were similar. All but one laboratory used either GC-FID or GC-MS following derivatization. The remaining laboratory used a liquid chromatography-tandem mass spectrometry method (LC-MS/MS) and reported data for only three fatty acids. For the GC analyses, the polarity range of the columns used was similar to those used in exercise 1 (Supplemental Table 1). For calibration, five laboratories used linear regression, four laboratories used quadratic regression, and one laboratory used a mix of linear and quadratic regression tailored by analyte. One laboratory reported only the limits of their calibration range, and three laboratories did not report calibration information.

3. Results and Discussion

With input from CDC and NIH-ODS, NIST contacted over 30 national and international laboratories in 2012 and over 40 in 2015 regarding participation in the first two FAQAP exercises. For the first exercise, 13 laboratories expressed interest and received samples, and for the second exercise, 15 laboratories expressed interest and received samples. Of those, 11 laboratories returned data for the 2012 exercise, and 14 laboratories returned data for the 2015 exercise. The participating laboratories are listed in alphabetical order in Table 1. In addition to multiple participants from the US, the exercises included participants from Canada, Korea, New Zealand, Australia, Portugal, and Thailand reflecting the international interest in quantifying individual fatty acids in human blood-based materials.

For the purposes of this discussion, the laboratories have been relabeled alphabetically (no relation to the alphabetical order in Table 1) with labs A through E participating only in the 2012 exercise, labs F through J participating in both exercises, and labs K through R participating in the 2015 exercise only. An additional laboratory, referred to as S, participated in both exercises; however, their data were not included in the summary

statistics because their data were consistently lower (between 80 % and 110 % lower) than the median in the first exercise and consistently higher (up to 2400 %) in the second exercise. This discussion will focus on a subset of the fatty acids quantified in both studies: two saturated fatty acids, C16:0 and C18:0; two monounsaturated fatty acids, C16:1n-7 and C18:1n-9; and four polyunsaturated fatty acids, C18:2n-6, C20:4n-6, C20:5n-3, and C22:6n-3.

3.1 Control Materials

For an assessment of the accuracy of the data received, control materials were provided for both exercises: SRM 1950 for the first exercise and the three levels of SRM 2378 for the second exercise. These materials were provided as knowns so the participants could compare their results to the values reported in the Certificates of Analysis (COA). The data received from the laboratories for the control materials were compared to the certified or reference values in Table 3. The relative expanded uncertainties at a 95% confidence interval of the certified and reference values for the indicated fatty acids in SRM 1950 and the three levels of SRM 2378 ranged from <1% to 18%. For labs F and I, NIST and CDC, respectively, the bias compared to the SRM values was generally small (−6% to 2% for NIST and −10% to −1% for CDC) and within the expanded uncertainties of the SRM values (Table 3). The bias in lab C's data showed a wide variation among the fatty acids ranging from −71% to 4%. For labs E, K, L, and M, the biases in their reported data were consistently lower compared to the SRM values for the majority of the fatty acids shown in Table 3. The biases in lab R's reported data were consistently higher (78% to 116%) than the SRM values for all fatty acids as shown in Table 3. Assuming this limited data set is representative of labs that participate in clinical studies, such discrepancies among the data from individual laboratories for individual fatty acids would make it difficult to compare data across different clinical studies.

Low values could be the result of incomplete extraction, and high values could be the result of coelution with other fatty acids or matrix components on the analytical column. Either low or high values could result from misidentification of the chromatographic peaks or inaccurate calibration. Supplemental Table 2 summarizes the analytical methods and quantification methods used by each laboratory. NIST (Lab F) used an acidified methanol derivatization followed by GC-FID analysis on a relatively polar column, and CDC (Lab I) used a pentafluorobenzyl bromide derivatization followed by GC-MS on a mid-polarity column (exercise 1) or a more polar column (exercise 2). The agreement of NIST and CDC's data both with the SRM values and between the two laboratories indicates that methods do not have to be the same to achieve agreement. One must validate their methods, however, with the use of appropriate control materials.

The precision of the data from the control materials was assessed as part of the first exercise in which participating laboratories were requested to analyze triplicate aliquots of SRM 1950. The data are presented in Supplemental Figure 1 as the relative difference from the COA values and associated expanded uncertainties along with the standard deviation for each participating laboratory's triplicate analyses. The precision of the laboratory data was relatively good and the bias was generally <20 %. However, for 7 of the 8 fatty acids shown,

the majority of the labs mean results exceeded the expanded uncertainties. For C20:5n3, no lab was within the expanded uncertainty.

3.2 Unknown Samples

Three unknown samples were distributed for each exercise, and participating laboratories were requested to do triplicate analyses for each sample. For each unknown in each study, the bias between the participating laboratory mean of the triplicate analyses and the median of the study results was calculated and is shown in Figure 1 for the selected fatty acids. The error bars shown in Figure 1 are the standard deviations for the biases from the median in each study. As indicated above, Lab S participated in both exercises but submitted data that were very different from the median data in the two exercises; therefore, their data were not included in the calculation of the median for either study.

The majority (70%) of the laboratory data agreed within 20% of the median of the exercises. However, lab R reported data that was > 50% higher than the median data, which is consistent with their high bias for control materials. Although the mean of lab G's data for the three unknowns in the second exercise agreed within 30% to the median of the data, the standard deviation of the biases showed a wide variation (up to 50%) indicating that they did not agree well with the median for each unknown. Lab H's data agreed closer to the median values in the second exercise than in the first exercise. Looking at Supplemental Table 2, the only reported difference between lab H's methods used in the two exercises was the addition of 28 external standards in the second exercise, possibly overcoming overestimation of recovery in the first exercise. Comparing the fatty acids shown in Figure 1, the largest variability both within laboratory and between laboratories was noted for C20:5n3, one of the long chain omega 3 PUFAs that are often of interest in clinical studies.

The spread among the data reported in the first two FAQAP exercises indicates the need for additional interlaboratory studies in this area as well as the need for laboratories to use control materials during their routine analyses, and SRMs in the development of their procedures. Intercomparison exercises provide an important mechanism for assessing the comparability, repeatability, and trueness of data being produced by the participating laboratories. Exercise materials similar in matrix, form, and analyte concentration to typical samples routinely analyzed by the laboratories are most useful for demonstrating the level of comparability and for revealing potential measurement and method problems. The data from these two exercises can be used to assess the comparability across a limited number of laboratories. The data indicate the need to increase the comparability of data across laboratories. Control materials are an important component for assessing the precision and accuracy of methods. The next exercise for the FAQAP exercise is scheduled for 2016 and annually thereafter.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Wu CL, Jain D, McNeill JN, Little D, Anderson JA, Huebner JL, Kraus VB, Rodriguiz RM, Wetsel WC, Guilak F. Dietary fatty acid content regulates wound repair and the pathogenesis of osteoarthritis following joint injury. *Ann Rheum Dis*. 2015; 74:2076–2083. [PubMed: 25015373]
2. Roy S, Brasky TM, Belury MA, Krishnan S, Cole RM, Marian C, Yee LD, Llanos AA, Freudenheim JL, Shields PG. Associations of erythrocyte-3 fatty acids with biomarkers of 3 fatty acids and inflammation in breast tissue. *Int J Cancer*. 2015; 137:2934–2946. [PubMed: 26137879]
3. Reinders U, Murphy RA, Song XL, Mitche GF, Visser M, Cotch MF, Garcia ME, Launer LJ, Eiriksdottir G, Gudnason V, Harris TB, Brouwers IA. Higher plasma phospholipid n-3 PUFAs but lower n- PUFAs are associated with lower pulse wave velocity among older adults. *J Nutr*. 2015; 145:2317–2324. [PubMed: 26311808]
4. Strandberg E, Edholm P, Ponsot E, Wahlin-Larsson B, Hellmen E, Nilsson A, Engfeldt P, Cederholm T, Riserus U, Kadi F. Influence of combined resistance training and healthy diet on muscle mass in healthy elderly women: a randomized controlled trial. *J Appl Physiol*. 2015; 119:918–925. [PubMed: 26338453]
5. Lu YH, Huang C, Zu YJ, Chia SE, Chen SS, Li N, Yu KK, Ling QX, Cheng Q, Zhu MQ, Chen MQ, Ong CN. Identification of serum biomarkers associated with hepatitis B virus-related hepatocellular carcinoma and liver cirrhosis using mass-spectrometry-based metabolomics. *Metabolomics*. 2015; 11:1526–1538.
6. Lankinen MA, Stancakova A, Uusituppa M, Agren J, Pihlajamaki J, Kuusisto J, Schwab U, Laakso M. Plasma fatty acids as predictors of glycaemia and type 2 diabetes. *Diabetologia*. 2015; 58:2533–2544. [PubMed: 26277381]
7. Kim SR, Jeon SY, Lee SM. The association of cardiovascular risk factors with saturated fatty acids and fatty acid desaturase indices in erythrocyte in middle-aged Korean adults. *Lipids Health Dis*. 2015; 14 Article 133.
8. Henriquez-Henriquez M, Solari S, Vargas G, Vasquez L, Allende F, Castanon C, Tenorio M, Guterrez TQ. 3-Long-chain polyunsaturated fatty acids and fatty acid desaturase activity ratios as eventual endophenotypes for ADHD. *J Atten Disord*. 2015; 19:977–986. [PubMed: 23100268]
9. Solberg DK, Bentsen H, Refsum H, Andreassen OA. Association between serum lipids and membrane fatty acids and clinical characteristics in patients with schizophrenia. *Acta Psychiatr Scand*. 2015; 132:293–300. [PubMed: 25597473]
10. Hennebell M, Harbeby E, Termblay S, Chouinard-Watkins R, Pifferi F, Plourde M, Guesnet P, Cunnane SC. Challenges in determining whether DHA can protect against age-related cognitive decline. *Clin Lipidol*. 2015; 10:91–102.
11. Phinney KW, Ballihaut G, Bedner M, Benford BS, Camara JE, Christopher SJ, Davis WC, Dodder NG, Eppe G, Lang BE, Long SE, Lowenthal MS, McGaw EA, Murphy KE, Nelson BC, Prendergast JL, Reiner JL, Rimmer CA, Sander LC, Schantz MM, Sharpless KE, Sniegowski LT, Tai SSC, Thomas JB, Veter TW, Welch MJ, Wise SA, Wood LJ, Guthrie WF, Hagwood CR, Leigh SD, Yen JH, Zhang NF, Caudhary-Webb M, Chen HP, Fazili Z, LaVoie DJ, McCoy LF, Momin SS, Paladugula N, Pendergast EC, Pfeiffer CM, Powers CD, Rabinowitz D, Rybak ME, Schleicher RL, Toombs BMH, Xu M, Zhang M, Castle AL. Development of Standard Reference Material for Metabolomics Research. *Anal Chem*. 2013; 85:11732–11738. [PubMed: 24187941]
12. Sander LC, Bedner M, Duewer DL, Lippa KA, Phillips MM, Phinney KW, Rimmer CA, Schantz MM, Sharpless KE, Tai SS-C, Thomas JB, Wise SA, Wood LJ, Betz JM, Coates PM. The Development and Implementation of Quality Assurance Programs to Support Nutritional Measurements. *Anal Bioanal Chem*. 2013; 405:4437–4441. [PubMed: 23552970]
13. Duewer DL, Brown Thomas J, Kline MC, MacCrehan WA, Schafer R, Sharpless KE, May WE, Crowell JA. NIST/NCI Micronutrients Measurement Quality Assurance Program: Measurement

- repeatabilities and reproducibilities for fat-soluble vitamin-related compounds in human sera. *Anal Chem.* 1997; 69:1406–1413. [PubMed: 9105181]
14. Duewer DL, Kline MC, Sharpless KE, Brown Thomas J, Gary KT, Sowell AL. Micronutrients Measurement Quality Assurance Program: Helping participants use interlaboratory comparison exercise results to improve their long-term measurement performance. *Anal Chem.* 1999; 71:1870–1878. [PubMed: 10330911]
 15. Duewer DL, Kline MC, Sharpless KE, Brown Thomas J. NIST Micronutrients Measurement Quality Assurance Program: Characterizing the measurement community's performance over time. *Anal Chem.* 2000; 72:4163–4170. [PubMed: 10994979]
 16. Duewer DL, Kline MC, Sharpless KE, Brown Thomas J, Stacewicz-Sapuntzakis M, Sowell AL. NIST Micronutrients Measurement Quality Assurance Program: Characterizing individual participant measurement performance over time. *Anal Chem.* 2000; 72:3611–3619. [PubMed: 10952550]
 17. Bedner M, Lippa KA, Tai SSC. An assessment of 25-hydroxyvitamin D measurements in comparability studies conducted by the Vitamin D Metabolites Quality Assurance Program. *Clin Chim Acta.* 2013; 426:6–11. [PubMed: 23978484]
 18. Schantz, MM.; Powers, CD.; Schleicher, R. Interlaboratory Analytical Comparison Study of Total Fatty Acid Concentrations in Human Serum: Results for Exercise 01: QA12FASER01. Gaithersburg, MD: 2013. NISTIR 7953
 19. Schantz, MM. Interlaboratory Analytical Comparison Study of Fatty Acid Concentrations in Human Serum: Results for Exercise 02: QA15FASER02. Gaithersburg, MD: 2015. NISTIR 8086

Figure 1.

Biases are the average of triplicate analyses of three unknowns in each exercise (exercise 1 – solid; exercise 2 – hashmark) compared to the median of participating laboratory measurements. Note that the data from one laboratory were not used to calculate the medians for each exercise.

Table 1

Participants in the two FAQAP exercises (in alphabetical order)

Laboratory	Participated in:	
	<u>Exercise 1 (2012)</u>	<u>Exercise 2 (2015)</u>
Bumrungrad Hospital Public Company Limited Bangkok THAILAND		x
Centers for Disease Control and Prevention (CDC) National Biomarkers Branch Atlanta, GA USA	x	x
Cornell University Division of Nutritional Sciences Ithaca, NY USA	x	
Craft Technologies Wilson, NC USA		x
Hanyang Univeristy Department of Food and Nutrition, College of Human Ecology Seoul KOREA	x	
Health Canada Nutrition Research Division Ottawa, ON CANADA		x
Lipid Analytical Labs Guelph, ON CANADA	x	
Matar Pathology Clinical Chemistry South Brisbane, Queensland AUSTRALIA		x
Mayo Clinic Biochemical Genetics Laboratory Rochester, MN USA	x	x
Minnesota Department of Health Public Health Laboratory Saint Paul, MN USA	x	x
National Institutes of Health LMBB, DICBR, NIAAA Rockville, MD USA	x	
National Institute of Standards and Technology (NIST) Chemical Sciences Division Gaithersburg, MD USA	x	x
OmegaQuant, LLC Sioux Falls, SD USA	x	
Polytechnic Institute of Bragança Escola Superior de Tecnologia e Gestão Bragança PORTUGAL		x
The University of Auckland Auckland Science Analytical Services, School of Biological Sciences Auckland NEW ZEALAND	x	x
University of California Los Angeles (UCLA) Center for Human Nutrition Los Angeles, CA USA		x
University of Michigan Cancer Center Ann Arbor, MI USA		x
University of Waterloo Department of Kinesiology Waterloo, ON Canada	x	x
USDA-ARS Western Human Nutrition Center Davis, CA USA		x

Table 2

List of suggested fatty acids in the FAQAP exercises

Code	Fatty Acid
C14:0	Myristic acid
C14:1n5	Myristoleic acid
C16:0	Palmitic acid
C16:1n7	Palmitoleic acid
C18:0	Stearic acid
C18:1n7	<i>cis</i> -Vaccenic acid
C18:1n9	Oleic acid
C18:2n6	Linoleic acid
C18:3n3	<i>alpha</i> -Linolenic acid
C18:3n6	<i>gamma</i> -Linolenic acid
C20:0	Arachidic acid
C20:1n9	11-Eicosenoic acid
C20:2n6	11,14-Eicosadienoic acid
C20:3n6	<i>homo-gamma</i> -Linolenic acid
C20:4n6	Arachidonic acid
C20:5n3	Eicosapentaenoic acid
C22:0	Docosanoic acid
C22:1n9	Docosenoic acid
C22:4n6	Docosatetraenoic acid
C22:5n3	Docosapentaenoic acid, n3
C22:5n6	Docosapentaenoic acid, n6
C22:6n3	Docosahexaenoic acid
C24:0	Lignoceric acid
C24:1n9	Nervonic acid

Table 3

Comparison of each laboratory's results to the certified or reference values for the control materials, SRM 1950 (2012 exercise) and SRM 2378-1, SRM 2378-2, and SRM 2378-3 (2015 exercise). For each control, the bias was calculated as the difference between the SRM Certificate of Analysis and the laboratory's reported value. Average difference ($\pm$ SD) was calculated for those laboratories that participated in the 2015 exercise. One lab that participated in both exercises was not included in the table due to large average biases.

		Saturated FA			Monounsaturated FA			Polyunsaturated FA					
		C16:0	C18:0		C16:1n-7	C18:1n-9		C18:2n-6	C20:4n-6		C20:5n-3		C22:6n-3
Relative Expanded Uncertainties for Controls ^a		$\pm 3.3\%$ to $\pm 18.0\%$	$\pm 6.0\%$ to $\pm 11.3\%$		$\pm 7.0\%$ to $\pm 12.1\%$	$\pm 7.1\%$ to $\pm 11.7\%$		$\pm 0.6\%$ to $\pm 17.1\%$	$\pm 6.0\%$ to $\pm 18.3\%$		$\pm 1.3\%$ to $\pm 13.0\%$		$\pm 4.0\%$ to $\pm 17.8\%$
Lab	# Controls	Bias	SD	Bias	SD	Bias	SD	Bias	SD	Bias	SD	Bias	SD
A	1	-7%		1%		26%		-1%		-36%		-3%	
B	1	7%		10%		26%		-8%		-36%		37%	
C	1	29%		45%		-71%		33%		4%		60%	
D	1	1%		12%		38%		3%		-29%		5%	
E	1	-30%		-18%		-14%		-27%		-45%		-7%	
F	4	-3%	7%	-2%	5%	-3%	6%	2%	8%	-6%	3%	0%	5%
G	4	-9%	25%	11%	18%	-7%	24%	-7%	9%	-9%	12%	12%	30%
H	4	10%	10%	19%	8%	20%	25%	9%	12%	-1%	10%	8%	13%
I	4	-4%	8%	-9%	3%	-5%	25%	19%	14%	-9%	10%	-3%	11%
J	4	-7%	8%	2%	8%	-5%	21%	-8%	9%	-16%	12%	-8%	12%
K	3	-36%	12%	-2%	5%	-48%	16%	-64%	30%	-30%	8%	3%	42%
L	3	-10%	6%	4%	4%	-14%	3%	-14%	3%	-12%	4%	-16%	3%
M	3	-14%	8%	31%	4%	-31%	2%	-26%	4%	-23%	6%	-47%	8%
N	3									70%	43%	0%	9%
O	3	16%	7%	18%	8%	24%	4%	13%	3%	47%	11%	17%	12%
P	3	-8%	4%	-4%	7%	-11%	18%	-15%	5%	-7%	6%	24%	40%
Q	3	-12%	8%	3%	8%	-16%	6%	-22%	3%	-11%	2%	3%	8%
R	3	85%	11%	109%	7%	78%	5%	107%	8%	116%	7%	112%	3%

^aThis row represents the range in the relative expanded uncertainties for the certified and reference values for the indicated fatty acids in SRM 1950 and the three levels of SRM 2378.