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NIST Fatty Acid Quality Assurance Program 2017 Final Report

Bruce A. Benner, Jr.
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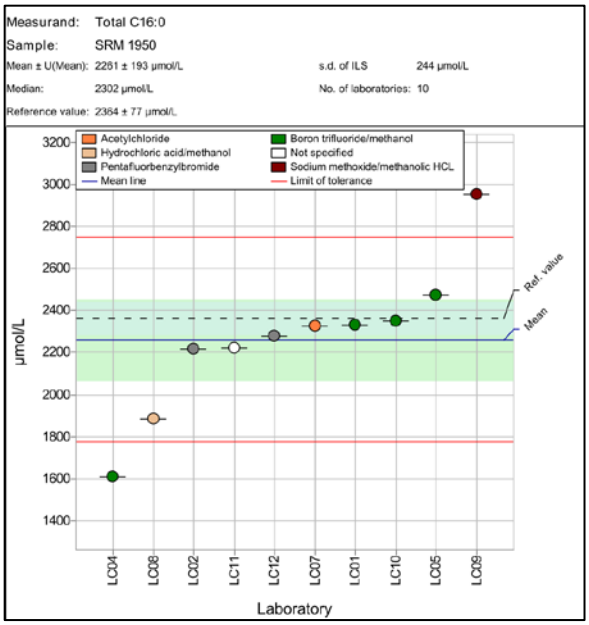

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Abstract

At the request of the National Institutes of Health (NIH) Office of Dietary Supplements (ODS) and in conjunction with the Centers for Disease Control and Prevention (CDC), in 2017 the National Institute of Standards and Technology (NIST) conducted the fourth Fatty Acid Quality Assurance Program (FAQAP) interlaboratory study of fatty acid (FA) concentrations in human serum. This 2017 FAQAP study included measurements of free FAs (extraction and measurement prior to acid/base hydrolysis) and total FAs (measurement after acid/base hydrolysis). Participants were requested to analyze SRM 1950 Metabolites in Frozen Human Plasma as a control, three "unknown" sera, and a free fatty acid (FFA) solution. Participants measuring fatty acid methyl ester (FAME) derivatives of FAs were asked to analyze a FAME solution. The results from this fourth exercise are reported along with a summary of the analytical methods used by the 14 participating laboratories.

Keywords

Fatty Acids (FAs), Free Fatty Acids (FFAs), Fatty Acid Methyl Esters (FAMEs)
Human Serum, Interlaboratory Comparison Study, SRM 1950

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Introduction

The fatty acid (FA) content of human plasma and serum can serve as indicators of health status and diet quality. Additionally, levels of FAs measured in their free form in serum or plasma, prior to any acid or base hydrolysis, versus conjugated as triglycerides and phospholipids, have been proposed as possible health markers for metabolic,[1] neurological,[2] and cardiovascular[3],[4] diseases. The National Institute of Standards and Technology (NIST) has offered the Fatty Acid Quality Assurance Program (FAQAP) through the NIST Clinical Quality Assurance Program (ClinQAP) since 2012. The first exercise of FAQAP was conducted in 2012 with 11 participants returning data for FAs in serum and plasma samples. [5] The second exercise of the FAQAP was conducted in 2015 with 14 participants returning data for FAs in serum samples. [6] The third exercise was conducted in 2016 with 13 participants reporting results for serum and plasma samples. [7]

This report summarizes the results from the 14 participants of the 2017 FAQAP. In this study, participants were asked to measure free and total FAs in three human sera samples (labeled as Unknown Serum 2017 A, B, and C, all three were the same serum material), SRM 1950 Metabolites in Frozen Human Plasma (control material), three free fatty acid solutions (labeled as Unknown Free FA Solution A, B, and C, all three were the same solution), and a fatty acid (FA)/fatty acid methyl ester (FAME) solution (labeled Unknown FA/FAME Sol D). Total FAs are defined as the fatty acid content after acid and/or base hydrolysis of the sample that converts the lipids into their individual fatty acid constituents. Free FAs are defined as those present inherently in the serum as unconjugated, endogenous free acids. Although, not the focus of the study, participants could also report free glycerol and total glycerides in the sera samples (Unknown Serum 2017 A, B, and C) and in the plasma control (SRM 1950). Samples were shipped to participants in March 2017 and results were returned to NIST in May 2017.

The 2017 FAQAP was the last NIST-conducted interlaboratory study devoted entirely to FAs and related analytes in human serum. The FA measurement community is now served through the Health Assessment Measurements Quality Assurance Program (HAMQAP). HAMQAP, in part a collaboration with the National Institutes of Health (NIH) Office of Dietary Supplements (ODS), represents NIST's ongoing and future support of the communities previously served by the Dietary Supplements QAP (DSQAP), Micronutrients Measurement QAP (MMQAP), Vitamin D Metabolites QAP (VitDQAP), as well as the FAQAP. NIST has decades of experience in the administration of these programs. HAMQAP builds on this experience by providing a wide range of matrices and analytes: nutritional and toxic elements, fat- and water-soluble vitamins, FAs, active and/or marker compounds, and contaminants. The HAMQAP design emphasizes emerging and challenging measurements in the dietary supplement, food, and clinical matrix categories.

Source of Materials

Study samples were provided to the participants free of charge. Participants received one ampoule of a control plasma material, SRM 1950, three ampoules of an unknown serum labeled as Unknown Serum 2017 Sample A, B, and C, three ampoules of an unknown free fatty acid solution labeled as Unknown Free FA Solution A, B, and C, and one ampoule of an unknown fatty acid/fatty acid methyl ester solution labeled as Unknown FA/FAME Solution D. Additional information of each of the materials is described below.

SRM 1950 Metabolites in Frozen Human Plasma [8].
Standard Reference Material (SRM) 1950 is a "normal" human plasma intended for validating methods for determining metabolites. The material has certified values for eight total FAs (Table 1) and reference values for an additional 19 total FAs (Table 2). A certified value is a value for which NIST has the highest confidence in its accuracy. A NIST reference value is a noncertified value that is the best estimate of the true value based on available data. The certified values were provided to the participants in the reporting sheet.

Table 1: Certified Values for Fatty Acids in SRM 1950

Lipid Name	Chemical Name / Common Name	Mass Fraction µg/g	Concentration µmol/L
C12:0	Dodecanoic Acid / Lauric Acid	1.86 ± 0.11	9.47 ± 0.57
C16:0	Hexadecanoic Acid / Palmitic Acid	594 ± 19	2364 ± 77
C16:1n7	(Z)-9-Hexadecenoic Acid / Palmitoleic Acid	53.5 ± 6.4	215 ± 26
C18:0	Octadecanoic Acid / Stearic Acid	179 ± 12	644 ± 41
C18:3n3	(Z,Z,Z)-9,12,15-Octadecatrienoic Acid / α-Linolenic Acid	14.9 ± 1.0	54.6 ± 3.6
C18:1n9	(Z)-9-Octadecenoic Acid / Oleic Acid	447 ± 43	1610 ± 150
C18:2n6	(Z,Z)-9,12-Octadecadienoic Acid / Linoleic Acid	780 ± 39	2840 ± 140
C22:0	Docosanoic Acid / Behenic Acid	15.9 ± 1.5	47.8 ± 4.6

Table 2: Reference Values for Fatty Acids in SRM 1950

Lipid Name	Chemical Name / Common Name	Mass Fraction µg/g	Concentration µmol/L
C14:0	Tetradecanoic Acid / Myristic Acid	17.9 ± 3.8	80 ± 17
C14:1	(Z)-9-Tetradecenoic Acid / Myristoleic Acid	1.57 ± 0.03	7.1 ± 0.1
C15:0	Pentadecanoic Acid	1.08 ± 0.01	4.56 ± 0.04
C17:0	Heptadecanoic Acid / Margaric Acid	4.7 ± 0.2	17.6 ± 0.7
C18:3n6	(Z,Z,Z)-6,9,12-Octadecatrienoic Acid / γ-Linolenic Acid	10.9 ± 2.3	39.9 ± 8.5
C18:1n7	(Z)-11-Octadecenoic Acid / *cis*-Vaccenic Acid	37.7 ± 0.9	136 ± 3
C20:0	Eicosanoic Acid / Arachidic Acid	5.5 ± 0.2	18.0 ± 0.5
C20:1	(Z)-11-Eicosenoic Acid / Gondolic Acid	3.5 ± 0.1	11.5 ± 0.5
C20:2	(Z,Z)-11,14-Eicosadienoic Acid	5.7 ± 0.2	18.8 ± 0.6
C20:3n6	(Z,Z,Z)-8,11,14-Eicosatrienoic Acid / Homo-γ-Linolenic Acid	41.8 ± 1.1	139 ± 4
C20:4n6	(Z,Z,Z,Z)-5,8,11,14-Eicosatetraenoic Acid / Arachidonic Acid	293 ± 54	980 ± 180
C20:5n3	(Z,Z,Z,Z,Z)-5,8,11,14,17-Eicosapentaenoic Acid / EPA	11.4 ± 0.1	38.6 ± 0.5
C22:1	(Z)-13-Docosenoic Acid / Erucic Acid	1.1 ± 0.4	3.4 ± 1.3
C22:4n6	(Z,Z,Z,Z)-7,10,13,16-Docosatetraenoic Acid	8.3 ± 0.2	25.5 ± 0.6
C22:5n3	(Z,Z,Z,Z,Z)-7,10,13,16,19-Docosapentaenoic Acid/ DPA	12.5 ± 0.2	38.5 ± 0.7
C22:5n6	(Z,Z,Z,Z,Z)-4,7,10,13,16-Docosapentaenoic Acid	6.3 ± 0.1	19.5 ± 0.4
C22:6n3	(Z,Z,Z,Z,Z,Z)-4,7,10,13,16,19-Docosahexaenoic Acid / DHA	37.9 ± 6.8	118 ± 21
C24:0	Tetracosanoic Acid / Lignoceric Acid	16.8 ± 0.9	46.6 ± 2.6
C24:1	(Z)-15-Tetracosenoic Acid / Nervonic Acid	25.6 ± 1.2	71.3 ± 3.2

Unknown Serum 2017.
This material was prepared from plasma obtained and processed into serum by a contractor in 2007. The serum was bottled in 1 mL aliquots and stored at -80 °C until shipment under dry ice. The approximate fatty acid concentrations (µmol/L) of the unknown serum material were not provided to the participants.

Unknown Free Fatty Acid Solution.
The unknown Free Fatty Acid Solution was prepared by diluting known amounts of nine free FAs in toluene. The solution was prepared at fatty acid levels that are anticipated in serum and plasma samples, and the gravimetric molar concentrations of nine FAs in the Unknown Free Fatty Acid Solution are listed in Table 3. The expanded uncertainty was approximated as 5 % of the mass fraction. Purity of the neat FAs used to prepare the Unknown Free Fatty Acid Solution was not determined. Since only free FAs were used in the solution preparation, the values for total and free FAs are the same (i.e., a method to determine total FAs should get the same value as a method to determine free FAs in this solution). The molar concentrations of the Unknown Free FA Solutions were unknown to the participants.

Table 3: Gravimetric Concentration of Free Fatty Acids in the Unknown FFA Solution.

Lipid Name	Chemical Name / Common Name	Concentration µmol/L
C18:0	Octadecanoic Acid / Stearic Acid	549 ± 28
C18:1n7	(Z)-11-Octadecenoic Acid / *cis*-Vaccenic Acid	115 ± 5.7
C18:1n9	(Z)-9-Octadecenoic Acid / Oleic Acid	1313 ± 66
C18:2n6	(Z,Z)-9,12-Octadecadienoic Acid / Linoleic Acid	2480 ± 124
C18:3n3	(Z,Z,Z)-9,12,15-Octadecatrienoic Acid / α-Linolenic Acid	44.4 ± 2.2
C20:4n6	(Z,Z,Z,Z)-5,8,11,14-Eicosatetraenoic Acid / Arachidonic Acid	804 ± 40
C20:5n3	(Z,Z,Z,Z,Z)-5,8,11,14,17-Eicosapentaenoic Acid / EPA	36.7 ± 1.8
C22:5n3	(Z,Z,Z,Z,Z)-7,10,13,16,19-Docosapentaenoic Acid / DPA	38.1 ± 1.9
C22:6n3	(Z,Z,Z,Z,Z,Z)-4,7,10,13,16,19-Docosahexaenoic Acid / DHA	98.4 ± 4.9

Unknown Fatty Acid/Fatty Acid Methyl Ester Solution.

The Unknown Fatty Acid/Fatty Acid Methyl Ester Solution was prepared by a collaborating laboratory and contains three FAs and 29 fatty acid methyl esters. The gravimetric molar concentration of each component in the solution is unknown to NIST and to the participants. Table 4 lists the FAs and the fatty acid methyl esters that are in the Unknown FA/FAME Solution. Since the solution contained both FAs and fatty acid methyl esters, participants were given the choice of reporting the molar concentration (µmol/L) either as the FA or as the FAME. Some participants reported molar concentrations for both.

Table 4: Fatty Acids and Fatty Acid Methyl Esters in the Unknown FA/FAME Solution.

Lipid Name	Analyte
C14:0	Myristic acid methyl ester
C14:1n5	Myristoleic acid methyl ester
C16:0	Palmitic acid methyl ester
C16:1n7	Palmitoleic acid methyl ester
C16:1n7t	Palmitelaidic acid methyl ester
C16:1n10	Sapienic acid
C16:1n5	*cis*-11-hexadecenoic acid
C16:1n5t	*trans*-11-hexadecenoic acid
C18:0	Stearic acid methyl ester
C18:1n7	*cis*-Vaccenic acid methyl ester
C18:1n9	Oleic acid methyl ester
C18:1n9t	Elaidic acid methyl ester
C18:1n7t	*trans*-vaccenic acid methyl ester
C18:1n12	Petroselinic acid methyl ester
C18:2n6	Linoleic acid methyl ester
C18:2n6t,9t	Linoelaidic acid methyl ester
C18:3n3	α-Linolenic acid methyl ester
C18:3n6	γ-Linolenic acid methyl ester
C20:0	Arachidic acid methyl ester
C20:1n9	11-Eicosenoic acid methyl ester
C20:2n6	11,14-Eicosadienoic acid methyl ester
C20:3n6	*homo*-γ-Linolenic acid methyl ester
C20:4n6	Arachidonic acid methyl ester
C20:5n3	Eicosapentaenoic acid methyl ester
C22:0	Docosanoic acid methyl ester
C22:1n9	Docosenoic acid methyl ester
C22:4n6	Docosatetraenoic acid methyl ester
C22:5n3	Docosapentaenoic acid methyl ester
C22:5n6	Docosapentaenoic acid methyl ester
C22:6n3	Docosahexaenoic acid methyl ester
C24:0	Lignoceric acid methyl ester
C24:1n9	Nervonic acid methyl ester

Instructions to Participants

Participants were instructed to analyze a single aliquot from each of the unknown serum samples (Unknown Serum 2017 A, B, and C), one aliquot of SRM 1950, a single aliquot from each of the unknown free fatty acid solutions (Unknown Free FA Sol. A, B, and C), and one aliquot of the unknown fatty acid methyl ester/free fatty acid solution (Unknown FAME/FFA sol. D) using their analytical protocols. Participants could report concentrations for total FAs, free FAs, free glycerol, and total triglycerides. Participants were not required to report values for all measurands listed on the reporting sheet and could add additional compounds when reporting data. Participants were asked to report values in µmol/L for FAs and mmol/L for glycerol and total triglycerides. The density of each material was provided for those converting from mass fraction to molar concentration. For the Unknown FA/FAME Sol., participants could either choose to report the molar concentration as FA, or FAME (when converting from mass fraction to molar concentration, results can be slightly different depending if the molecular weight of the FA or FAME is used). Some participants reported results for both, while others reported results for one or the other. No calculations were done to convert one from the other after results were reported by the participants. The data file template given to participants for reporting data included multiple sheets for the participants to list information on analytical methods as well as results.

Methods

Participants were asked to use analytical protocols currently used in their laboratory. For total fatty acid analysis, there were several participants that performed base hydrolysis with BF_3/methanol derivatization, as well as acid/base hydrolysis and pentafluorobenzylbromide derivatization. Table 5 summarizes the method details for total FAs.

Four participants reported results for free FAs using different measurement protocols. Table 6 summarizes the method details for free FAs. The method summary for the analysis of the fatty acid solutions (Unknown FA Sol. and unknown FA/FAME Sol.) is shown in Table 7. Two participants reported total glycerides and the summary of the methods used is in Table 8. Table 9 shows the summary of the laboratory that reported free glycerol. Full detail method descriptions as reported by each laboratory can be found in Appendix D.

Table 5: Summary of Participant's Methods for Total Fatty Acids in Serum.

Code	Sample Preparation Method	Analytical Method
LC01	BF3/methanol derivatization	GC-FID on non-bonded poly(biscyanopropyl siloxane)
LC02	Acid/base hydrolysis and pentafluorbenzylbromide derivatization	GC-MS on 5 % phenyl-95 % methylpolysiloxane
LC04	BF3/methanol derivatization	GC-FID on FFAP phase column
LC05	BF3/methanol derivatization	GC-FID on FFAP phase column
LC07	Extraction and acetylchloride derivatization	GC-FID on (88 %-cyanopropyl)aryl-polysiloxane
LC08	Acid hydrolysis and methanol	GC-MS on 5 % phenyl-95 % methylpolysiloxane
LC09	Dry methanolic sodium methoxide plus methanolic HCL	GC-MS on (50 % cyanopropylphenyl)-dimethylpolysiloxane
LC10	BF3/methanol derivatization	GC-FID on (50 %-cyanopropyl)-methylpolysiloxane
LC11	Liu et al. 2010	GC-MS on 5 % phenyl-95 % methylpolysiloxane
LC12	Acid/base hydrolysis and pentafluorbenzylbromide derivatization	GC-MS on a select FAME column
LC14	Acid/base hydrolysis and pentafluorbenzylbromide derivatization	GC-MS on TG-Polar phase

Table 6: Summary of Participant's Methods for Free Fatty Acids in Serum.

Code	Sample Preparation Method	Analytical Method
LC01	BF3 in Methanol/hexane cold temperature	GC-FID on non-bonded poly(biscyanopropyl siloxane)
LC05	BF3/methanol derivatization after FFA separation by thin layer chromatography	GC-FID on FFAP phase column
LC06	Liquid/liquid extraction and no derivatization	GC-MS on FFAP column
LC09	Extraction and (trimethylsilyl)diazomethane derivatization	GC-MS on (50 % cyanopropylphenyl)-dimethylpolysiloxane

Table 7: Summary of Participant's Methods for Solutions

Code	Sample Preparation Method	Analytical Method
LC01	BF3/methanol derivatization	GC-FID on non-bonded poly(biscyanopropyl siloxane)
LC02	Acid/base hydrolysis and pentafluorbenzylbromide derivatization	GC-MS on 5 % phenyl-95 % methylpolysiloxane
LC04	BF3/methanol derivatization	GC-FID on FFAP phase column
LC05	BF3/methanol derivatization	GC-FID on FFAP phase column
LC07	extraction and acetylchloride derivatization	GC-FID on (88 %-cyanopropyl)aryl-polysiloxane
LC08	Acid hydrolysis and methanol	GC-MS on 5 % phenyl-95 % methylpolysiloxane
LC09	Dilute and (trimethylsilyl)diazomethane derivatization	GC-MS on (50 % cyanopropylphenyl)-dimethylpolysiloxane
LC10	BF3/methanol derivatization	GC-FID on (50 %-cyanopropyl)-methylpolysiloxane
LC11	Liu et al. 2010	GC-MS on 5 % phenyl-95 % methylpolysiloxane
LC12	Acid/base hydrolysis and pentafluorbenzylbromide derivatization	GC-MS on a select FAME column
LC14	Acid/base hydrolysis and pentafluorbenzylbromide derivatization	GC-MS on TG-Polar phase

Table 8: Summary of Participant's Methods for Total Glycerides in Serum.

Code	Sample Preparation Method	Analytical Method
LC03	Base hydrolysis and pyridine and acetic anhydride derivatization	GC-MS on 50 % phenly-methylpolysiloxane
LC13	Base hydrolysis and pyridine and acetic anhydride derivatization	GC-MS on 50 % phenly-methylpolysiloxane

Table 9: Summary of Participant Method for Free Glycerol in Serum

Code	Sample Preparation Method	Analytical Method
LC13	Ethanol precipitation and pyridine and acetic anhydride derivatization	GC-MS on 50 % phenly-methylpolysiloxane

Overview of Data Treatment and Representation

Data tables and graphs are provided throughout this report using anonymized laboratory codes.

Statistics

Data tables and graphs throughout this report contain information about the relative performance of each laboratory. All calculations were performed in PROLab Plus (QuoData GmbH, Dresden, Germany). The consensus mean and standard deviation are calculated according to the robust Q/Hampel method outlined in ISO 13528:2015(E), Annex C.

Data Tables

The data submitted by the participants, listed by lab code, are detailed in Appendix A for free and total FAs in plasma and serum samples (Unknown Serum 2017 samples A, B, and C, and SRM 1950), Appendix B for FAs and fatty acid methyl esters in the solutions (Unknown Free FA Sol A, B, and C and Unknown FA/FAME Sol. D), and Appendix C for free glycerol and total glycerides in plasma and serum samples. The results are organized by analyte (indicated on the top line of each table) The laboratory average and standard deviation for samples with multiple measurements (unknown serum 2017 samples and unknown Free Fatty Acid Sol.) are listed. Community statistics are summarized at the bottom of each sample column, including the consensus mean and standard deviation calculated by the robust Q/Hampel method and the number of participants determined in the analysis. Blank spaces in the data table next to a laboratory indicate that that laboratory did not return data for that analyte-sample combination. NIST values listed for SRM 1950 are the certified or reference values and their associated uncertainties as listed in the Certificate of Analysis.[8]

Graphical Representation:

For each analyte-sample combination when two or more participants reported results, a summary page was generated. At the top of each summary page is the measurand and sample name in bold. Below this information is the consensus mean and uncertainty that was determined using the robust Q/Hampel method. The median is also listed below the consensus mean. If available, the reference value is listed with its associated uncertainty. This reference value is either the certified or reference value of SRM 1950 or the gravimetric value of the Unknown Free Fatty Acid Solution. The robust standard deviation is listed on the right along with the number of participants in the calculation.

Below this information in the summary page is a graphical representation of the data. In this view, individual laboratory data (circles) are plotted with the individual laboratory standard deviations (rectangles). The blue solid line represents the consensus mean, and the green shaded area represents the 95 % confidence interval for the consensus mean, based on the standard error of the consensus mean. The uncertainty in the consensus mean is calculated using the equation below (ISO 13528), based on the robust standard deviation (s*, listed as s.d. of ILS at the top of each summary page) and the number of participants reporting data (n).

$$u_{mean} = 1.25 \frac{s^*}{\sqrt{n}}$$

The black dotted line is the reference value (when available). The blue shaded region represents the expanded uncertainty of the reference value. The solid red lines represent the range of tolerance (values that result in an acceptable Z score, $|Z| \leq 2$). If the lower limit is below zero, the lower limit has been set to zero. In this view, the relative locations of individual laboratory data and consensus zones with respect to the reference zone can be compared easily. In most cases, the target zone and the consensus zone overlap, which is the expected result. The results are color coded based on the analytical method used, a short description of the analytical methods is indicated in the legend of the plot. This can be used to determine if there are method biases.

Results and Discussion

Total Fatty Acids in SRM 1950:

SRM 1950 was included in the study as a control. Eight total FAs are certified in SRM 1950 and an additional 19 total FAs have reference values (noncertified values). The certified values were listed on the reporting sheet, and hence participants knew the certified values before submitting results. The data tables and the graphical representation summarizing the results submitted by the participants for SRM 1950 are in Appendix A. There was good agreement for most total FAs when comparing the consensus range, the 95 % confidence level of the consensus mean (the green shaded area in the graphical representation of the data), with the certified or reference range. This along with the 95 % confidence level (the blue shaded area in the graphical representation of the data), indicates that the consensus values obtained in this study are comparable to the certified or reference values of SRM 1950. The consensus range for 23 out of 25 FAs overlapped with the certified or reference range (see Table 10). This can be easily viewed in the graphical representation when the green and blue shaded areas overlap. Of these 23 FAs, the consensus mean for eight FAs were within the certified or reference range (indicated when the blue solid line that represents the mean lies within the blue shaded region in the graphs). However, for some FAs the uncertainty of the consensus mean was large. This could be due to large variability in the data returned and/or the low number of labs returning data since the uncertainty is inversely proportional to the square root of the number of participants.

The consensus range was outside the reference range for both total pentadecanoic acid (C15:0) and total arachidonic acid (C20:4 n-6) in SRM 1950. It is important to note that for both of these FAs, only a reference (noncertified) value is available in SRM 1950, which may not capture all sources of bias in the uncertainty.

The between-laboratory variability, estimated as the percent relative standard deviation (RSD), for the total FAs that have certified or reference values in SRM 1950 is also summarized in Table 10. The RSD was low for most FAs (less than 30 %), though ten FAs measured yielded RSDs > 30 % and three FAs had RSDs > 60 % for this control material. The largest RSDs were typically for FAs with low molar concentrations. Between-laboratory variability improved with higher molar concentration analytes.

Table 10: Comparison of Consensus Mean and Range to SRM 1950 Total FA Values[a]

Lipid	Common Name	SRM 1950 Certified?	Consensus Mean	Consensus Range	RSD [c] %
C12:0	Lauric acid	Yes	Below	NA[b]	91
C14:0	Myristic Acid	No	Within	Overlaps	30
C14:1n5	Myristoleic Acid	No	Above	Overlaps	67
C15:0	Pentadecanoic Acid	No	Above	Above	60
C16:0	Palmitic Acid	Yes	Below	Overlaps	11
C16:1n7	Palmitoleic Acid	Yes	Above	Overlaps	11
C17:0	Margaric Acid	No	Below	Overlaps	45
C18:0	Stearic Acid	Yes	Within	Overlaps	25
C18:1n7	*cis*-Vaccenic Acid	No	Above	Overlaps	22
C18:1n9	Oleic Acid	Yes	Within	Overlaps	16
C18:2n6	Linoleic Acid	Yes	Within	Overlaps	14
C18:3n3	α-Linolenic Acid	Yes	Below	Overlaps	26
C18:3n6	γ-Linolenic Acid	No	Within	Overlaps	25
C20:0	Arachidic Acid	No	Above	Overlaps	27
C20:1n9	11-Eicosenoic acid	No	Below	Overlaps	54
C20:2n6	11,14-Eicosadienoic acid	No	Below	Overlaps	40
C20:3n6	homo-γ-Linolenic acid	No	Below	Overlaps	31
C20:4n6	Arachidonic Acid	No	Below	Below	32
C20:5n3	EPA	No	Below	Overlaps	55
C22:0	Docosanoic acid	Yes	Within	Overlaps	24
C22:4n6	Docosatetraenoic acid	No	Below	Overlaps	25
C22:5n3	DPA	No	Within	Overlaps	8
C22:5n6	Docosapentaenoic acid	No	Below	Overlaps	23
C22:6n3	DHA	No	Within	Overlaps	24
C24:0	Lignoceric acid	No	Below	Overlaps	32

[a] Interlaboratory Relative Standard Deviation: RSD = 100*SD/Mean.

[b] Not applicable as the RSD for this FA is too large.

[c] Comparison between expanded uncertainties of the certificate values with standard deviations of the means for the results from the participating laboratories.

Total Fatty Acids in Unknown Serum:

Two to eleven participants reported results for 38 total FAs in Unknown serum A, B, and C, which were the same material (see Appendix A). The replicates of the unknown Serum 2017 study are plotted for each laboratory in the graphical representation in Appendix A (individual laboratory data points are represented by circles, with the individual laboratory standard deviations represented by the rectangles). While for most participants and FAs, the intralaboratory repeatability is good, the interlaboratory variability for many of the FAs in the Unknown Serum 2017 samples are large (Table 11). There are 12 total FAs with a RSD of less than 30 %, and 26 total FAs with a RSD greater than 30 %, and 12 of the 26 FAs have RSD greater than 60 %. Similar to SRM 1950, many of the FAs in the unknown serum samples that have high variability have low molar concentrations and/or a low number of participants returning data. It is difficult to draw conclusions from data for analytes with low participation rates. Robust statistics does not work well with a low number of participants, and hence, outlier results may influence the consensus mean and standard deviation more compared to when larger number of participants report results. Generally good agreement was observed for the total FAs measured by most participants. Out of the 38 total FAs in Table 11, eight or more participants submitted data for 19 FAs. Out of these 19 FAs, 11 of the FAs had RSD below 30 %.

There is both large interlaboratory variability and low participation rates for the trans FAs. For example, four participants reported results for elaidic acid (C18: 1n9t); two participants reported molar concentrations higher than 100 µmol/L, one laboratory reported a molar concentration around 40 µmol/L, and the fourth reported a molar concentration of 1 µmol/L. One possible reason for the high variability is due to inadequate separation of the trans FAs from other components. Other reasons of high variability in the data could be due to method biases, such as incomplete hydrolyses of the triglycerides and phospho-lipids containing the FAs.

Table 11: Total Fatty Acids in Unknown Serum

Lipid	Common Name	*N*	Mean ± SD µmol/L	RSD %
C10:0	Decanoic acid	4	7.0 ± 8.1	117
C12:0	Lauric acid	6	15 ± 16	103
C12:1n1	11-Dodecenoic acid	2	2.7 ± 1.5	55
C14:0	Myristic acid	10	121 ± 43	36
C14:1n5	Myristoleic acid	7	8.5 ± 8.7	102
C15:0	Pentadecanoic acid	6	20 ± 14	72
C16:0	Palmitic acid	10	2699 ± 445	16
C16:1n7	Palmitoleic acid	11	236 ± 49	21
C16:1n7t	Palmitelaidic acid	3	22 ± 22	102
C17:0	Margaric acid	5	27 ± 16	59
C18:0	Stearic acid	11	829 ± 169	20
C18:1n7	cis-Vaccenic acid	7	171 ± 57	33
C18:1n9	Oleic acid	11	2103 ± 430	20
C18:1n9t	Elaidic acid	4	77 ± 140	182
C18:2n6	Linoleic acid	11	3433 ± 726	21
C18:2n6t,9t	Linoelaidic acid	5	70 ± 80	113
C18:3n3	α-Linolenic acid	11	56 ± 20	35
C18:3n6	γ-Linolenic acid	11	39 ± 22	56
C18:4n3	Stearidonic acid	3	4.3 ± 4.4	102
C19:0	Nonadecanoic acid	2	2.8 ± 8.9	314
C20:0	Arachidic acid	9	25.4 ± 8.3	33
C20:1n9	11-Eicosenoic acid	9	11.3 ± 6.0	53
C20:2n6	11,14-Eicosadienoic acid	9	20.2 ± 8.6	42
C20:3n3	11,14,17-Eicosatrienoic acid	2	4.6 ± 7.4	161
C20:3n6	homo-γ-Linolenic acid	11	152 ± 30	20
C20:3n9	5,8,11-Eicosatrienoic acid	3	11.0 ± 6.8	62
C20:4n6	Arachidonic acid	11	681 ± 110	16
C20:5n3	Eicosapentaenoic acid	10	44 ± 19	42
C22:0	Docosanoic acid	8	61 ± 22	37
C22:1n9	Docosenoic acid	6	7.5 ± 8.0	107
C22:2n6	Docosadienoic acid	2	2.7 ± 1.4	52
C22:4n6	Docosatetraenoic acid	9	23.2 ± 4.3	19
C22:5n3	Docosapentaenoic acid	9	41.3 ± 3.0	7
C22:5n6	Docosapentaenoic acid	9	26.6 ± 7.9	30
C22:6n3	Docosahexaenoic acid	10	168 ± 20	12
C23:0	Tricosanoic acid	4	20 ± 14	70
C24:0	Lignoceric acid	8	47 ± 13	27
C24:1n9	Nervonic acid	8	71 ± 10	14

Free Fatty Acids in SRM 1950 and Unknown Serum A, B, C:

There was a low number of labs returning data for free FAs in the SRM 1950 and Unknown Serum 2017 samples (see Appendix A for data tables and graphical representation). Table 12 lists the consensus mean and standard deviation of free FAs determined in both SRM 1950 and in the Unknown Serum 2017 samples. Free FAs with the largest standard deviations were typically observed for the FAs present at lower molar concentrations. It is interesting to note that different methods were used by the four participants that reported free FAs. Most of the participants took care to avoid exposing the samples to elevated temperatures during the extraction steps, so as not to induce deconjugation of the FAs from triglycerides or phospholipids.

Table 12: Consensus Mean of Select Free Fatty Acids in SRM 1950 And Unknown Serum

		SRM 1950		Unknown Serum	
Lipid	Common Name	*N*	Mean ± SD µmol/L	*N*	Mean ± SD µmol/L
C14:0	Myristic acid	4	8.0 ± 1.3	4	9.3 ± 1.4
C16:0	Palmitic acid	4	156 ± 91	4	178 ± 16
C16:1n7	Palmitoleic acid	4	17.4 ± 3.6	4	14.0 ± 2.6
C18:0	Stearic Acid	4	32 ± 36	4	46 ± 34
C18:1n7	*cis*-Vaccenic Acid	2	16 ± 12	3	10 ± 14
C18:1n9	Oleic Acid	4	149 ± 30	4	133 ± 18
C18:2n6	Linoleic Acid	4	70 ± 43	4	109 ± 20
C18:3n3	α-Linolenic Acid	2	3.9 ± 2.0	2	3.4 ± 1.8
C18:3n6	γ-Linolenic Acid	3	0.997 ± 0.025	3	0.86 ± 0.38
C20:3n6	homo-γ-Linolenic acid	3	1.3 ± 1.0	3	2.2 ± 2.0
C20:4n6	Arachidonic acid	3	7.2 ± 6.9	3	12.8 ± 6.0
C20:5n3	EPA (C20:5 n-3)	2	0.48 ± 0.50	3	0.92 ± 1.0
C22:4n6	Docosatetraenoic acid	2	0.83 ± 0.36	3	0.45 ± 0.61
C22:5n3	DPA	3	1.25 ± 1.4	3	0.79 ± 0.38
C22:5n6	Docosapentaenoic acid	2	0.546 ± 0.080	3	0.54 ± 0.41
C22:6n3	DHA	3	2.4 ± 2.4	3	3.8 ± 1.4

Fatty Acid Solutions A,B,C:

Eight to twelve participants reported results for the Unknown Fatty Acid Sol. A, B, and C, which were of the same material (See Appendix B). The consensus range (95 % confidence interval of the consensus mean) overlapped with the gravimetric value with an estimated expanded uncertainty of 5 % of the gravimetric value for all FAs. All but two consensus mean values fell within the estimated gravimetric uncertainty except for EPA and DPA. The interlaboratory variability for the unknown fatty acid sol. for all FAs but EPA were less than 30 %.

Table 13: Comparison of Consensus Ranges to Gravimetric Target in Unknown FFA Solution

Lipid	Common Name	Consensus Mean	Consensus Range	RSD %
C18:0	Stearic Acid	within	overlaps	17
C18:1n7	*cis*-Vaccenic Acid	within	overlaps	19
C18:1n9	Oleic Acid	within	overlaps	17
C18:2n6	Linoleic Acid	within	overlaps	15
C18:3n3	α-Linolenic Acid	within	overlaps	22
C20:4n6	Arachidonic Acid	Within	overlaps	24
C20:5n3	EPA	below	overlaps	34
C22:5n3	DPA	Slightly below	overlaps	13
C22:6n3	DHA	within	overlaps	17

Unknown Fatty Acid/Fatty Acid Methyl Ester Solution D:

The Unknown Fatty Acid/Fatty Acid Methyl Ester Sol. consisted of three FAs and 29 fatty acid methyl esters. Since the solution contained both FAs and fatty acid methyl esters, participants were given the choice of reporting the molar concentration either as the fatty acid or as the fatty acid methyl ester. The choice was given to the participants in case participants were converting mass fraction values to molar concentration. However, the choice of reporting the molar concentration as the FA or the FAME confused some of the participants. Some participants reported the molar concentration as the FA or the FAME, while some participants reported both (see Appendix B). For the participants that reported results for both, it was unclear how these values were determined. For at least one laboratory, the results before and after methylation were reported as the FAME and FA respectively.

Two to four participants reported results for the Unknown Fatty Acid/FAME Sol. as FAME. Results for nine selected FAs in Table 14 suggest generally good agreement between the labs with all RSD less than 30 % except for arachidonic acid (43 %). Six to four participants reported molar concentration of the Unknown Fatty Acid/FAME Sol. as µmol/L of the FA. The selected nine analytes had RSD of less than 30 %, indicating good between lab agreement.

Table 14: Consensus Mean of Fatty Acid Methyl Esters in FAME Solution D

		Reported as FAME		Reported as FA	
Lipid	Common Name	*N*	Mean ± SD µmol/L	*N*	Mean ± SD µmol/L
C18:0	Stearic Acid	4	559 ± 29	8	534 ± 80
C18:1n7	*cis*-Vaccenic Acid	2	220 ± 37	6	216 ± 59
C18:1n9	Oleic Acid	4	1814 ± 360	8	1647 ± 246
C18:2n6	Linoleic Acid	4	2239 ± 457	8	2082 ± 229
C18:3n3	α-Linolenic Acid	4	100 ± 28	7	112 ± 3.2
C20:4n6	Arachidonic Acid	4	469 ± 202	8	435 ± 50
C20:5n3	EPA	4	223 ± 38	7	190 ± 37
C22:5n3	DPA	3	53.3 ± 7.4	7	52.5 ± 5.8
C22:6n3	DHA	4	310 ± 45	7	279 ± 44

Total Glycerides and Free Glycerol in SRM 1950 and Unknown Serum 2017:

Two participants reported values for total glycerides for SRM 1950 and for Unknown Serum 2017 Samples (see Appendix C). The consensus mean (1.10 mmol/L ± 0.03 mmol/L) for total glyceride in SRM 1950 is comparable to the certified value in SRM 1950 (1.12 mmol/L ± 02 mmol/L). The two participants also reported similar values for total glycerides in the Unknown Serum 2017 samples as well (1.53 mmol/L and 1.54 mmol/L). Only one of these participants also reported a value for free glycerol.

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Appendix A: Free and Total Fatty Acids in Plasma and Serum

List of Tables

Acronyms Used in Tables

Avg	Mean
FA	Total fatty acid
FFA	Free fatty acid
Lab	Participant code
<LOD	Less than the limit of detection
N	Number of quantitative results
SD	Standard deviation

Table A-1: C16:1n5 *cis*-11-hexadecenoic acid, µmol/L

	FA	2017 Unknown, FA					FFA	2017 Unknown, FFA				
Lab	SRM 1950	A	B	C	Avg	SD	SRM 1950	A	B	C	Avg	SD
LC05	6.80	9.04	8.32	8.63	8.67	0.36	0.578					

Table A-2: C16:1n5t *trans*-11-Hexadecenoic acid, µmol/L

	FA	2017 Unknown, FA					FFA	2017 Unknown, FFA				
Lab	SRM 1950	A	B	C	Avg	SD	SRM 1950	A	B	C	Avg	SD
LC05	6.92	12.4	11.6	11.9	12.0	0.40	0.969	1.06	1.39	1.24	1.23	0.17

Table A-3: C16:1n10 Sapienic acid, µmol/L

	FA	2017 Unknown, FA					FFA	2017 Unknown, FFA				
Lab	SRM 1950	A	B	C	Avg	SD	SRM 1950	A	B	C	Avg	SD
LC05	49.0	53.6	54.6	54.7	54.3	0.57						

Table A-4: C18:1n7t *trans*-Vaccenic acid, µmol/L

	FA	2017 Unknown, FA					FFA	2017 Unknown, FFA				
Lab	SRM 1950	A	B	C	Avg	SD	SRM 1950	A	B	C	Avg	SD
LC12	20.4	35.4	36.0	35.5	35.6	0.33						

Table A-5: C21:0 Heneicosanoic acid, µmol/L

	FA	2017 Unknown, FA					FFA	2017 Unknown, FFA				
Lab	SRM 1950	A	B	C	Avg	SD	SRM 1950	A	B	C	Avg	SD
LC05	2.09	3.02	3.55	2.81	3.13	0.38	0.201	0.286	0.225	0.180	0.230	0.053

Table A-6: C10:0 Decanoic acid, µmol/L

	FA		2017 Unknown, FA					FFA	2017 Unknown, FFA				
Lab	SRM 1950		A	B	C	Avg	SD	SRM 1950	A	B	C	Avg	SD
LC02			8.00	8.00	8.00	8.00	0.00						
LC05		11.5	11.1	11.7	14.4	12.37	1.8						
LC08		1.85	3.21	3.49	3.19	3.30	0.17						
LC10	<LOD		<LOD	<LOD	<LOD	<LOD							
LC14		2.61	4.14	4.25	4.00	4.13	0.13						
	Avg	5.32				Avg	6.95						
	SD	6.2				SD	8.1						
	N	3				N	4						

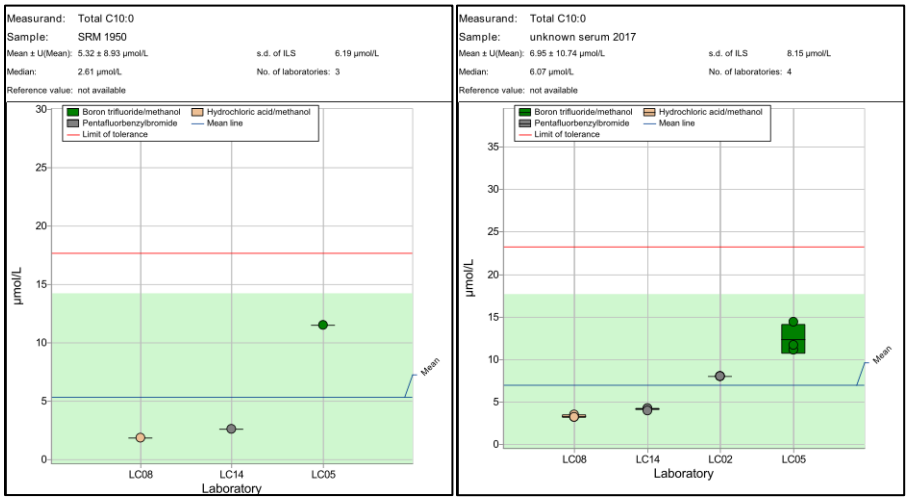
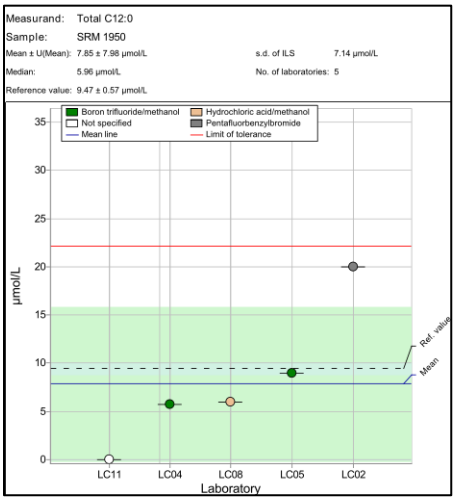


Table A-7: C12:0 Lauric acid, µmol/L

	FA	2017 Unknown, FA					FFA	2017 Unknown, FFA				
Lab	SRM 1950	A	B	C	Avg	SD	SRM 1950	A	B	C	Avg	SD
NIST	9.47 ± 0.57											
LC02	20.0	31.0	30.0	30.0	30.33	0.58						
LC04	5.75	15.2	17.8	11.8	14.9	3.0						
LC05	8.97	16.9	16.7	16.8	16.8	0.10						
LC08	5.96	10.2	9.48	9.56	9.74	0.38						
LC10	<LOD	19.1	<LOD	<LOD	19.1							
LC11	0	0.89	0.56	0.91	0.79	0.20						
	Avg 7.85				Avg 15.28							
	SD 7.1				SD 15.8							
	N 5				N 6							



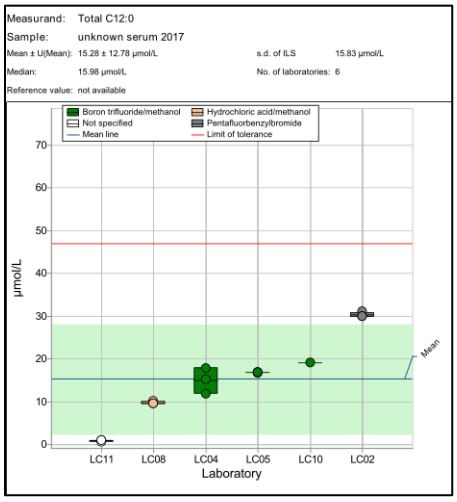


Table A-8: C12:1n1 11-Dodecenoic acid, µmol/L

	FA	2017 Unknown, FA					FFA	2017 Unknown, FFA				
Lab	SRM 1950	A	B	C	Avg	SD	SRM 1950	A	B	C	Avg	SD
LC02		2.9	2.8	2.8	2.83	0.06						
LC05	1.756	1.69	2.22	3.72	2.54	1.05						
					Avg	2.69						
					SD	1.5						
	N 1				N	2						

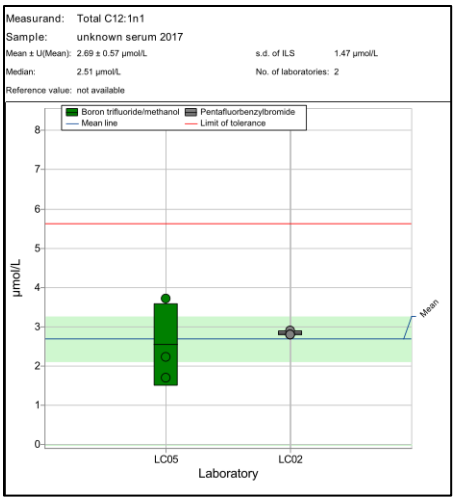
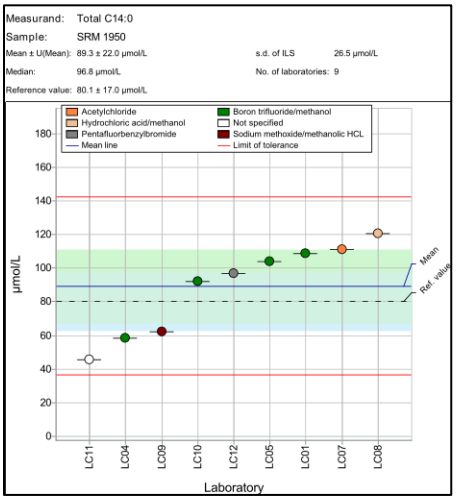
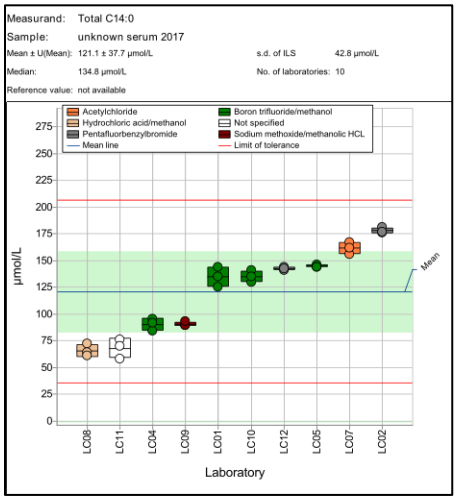
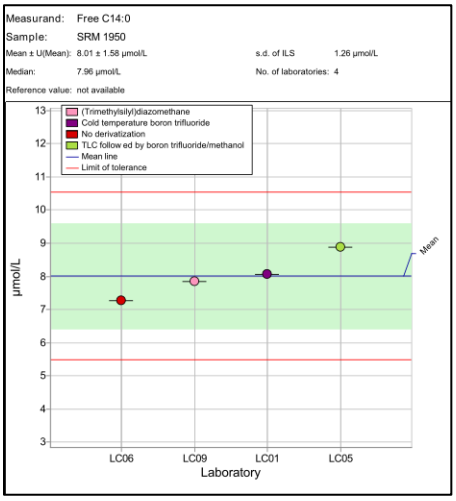


Table A-9: C14:0 Myristic acid, µmol/L

	FA		2017 Unknown, FA					FFA		2017 Unknown, FFA				
Lab	SRM 1950		A	B	C	Avg	SD	SRM 1950		A	B	C	Avg	SD
NIST	80 ± 17													
LC01		109	125	144	135	135	9.3		8.07	8.33	8.07	7.98	8.13	0.18
LC02			181	176	177	178	2.6							
LC04		58.2	84.1	95.2	91.3	90.2	5.7							
LC05		104	146	144	144	145	1.1		8.87	10.1	10.2	10.4	10.2	0.16
LC06									7.27	9.37	8.39	7.80	8.52	0.79
LC07		111	167	156	162	162	5.5							
LC08		121	72.0	63.9	60.8	65.5	5.8							
LC09		62.4	89.4	89.1	93.0	90.5	2.1		7.84	9.91	9.58	10.9	10.1	0.69
LC10		92.2	141	130	135	135	5.5							
LC11		45.7	57.8	75.9	70.3	68.0	9.3							
LC12		96.8	141	144	142	142	1.4							
	Avg	89.3				Avg	121	Avg	8.01				Avg	9.25
	SD	26				SD	43	SD	1.3				SD	1.3
	N	9				N	10	N	4				N	4







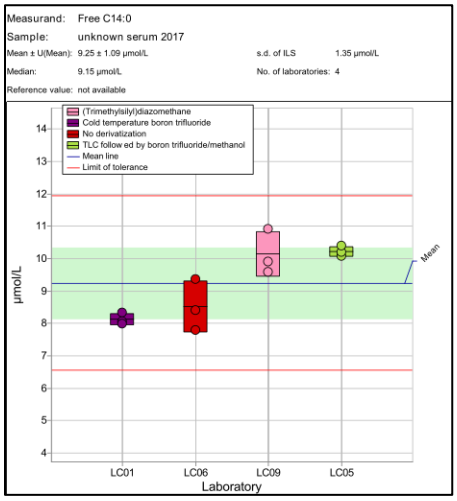
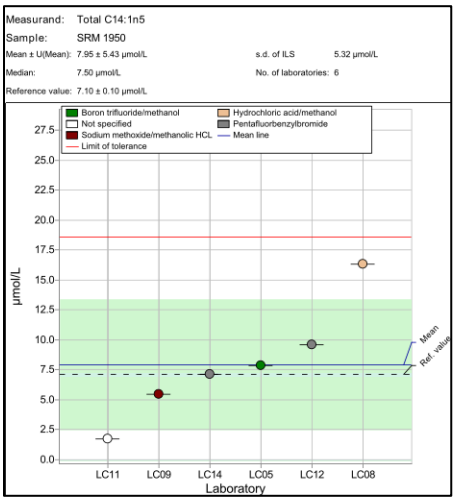


Table A-10: C14:1n5 Myristoleic acid, µmol/L

	FA		2017 Unknown, FA					FFA	2017 Unknown, FFA				
Lab	SRM 1950		A	B	C	Avg	SD	SRM 1950	A	B	C	Avg	SD
NIST	7.1 ± 0.1												
LC02			21.0	21.0	21.0	21.0	0.00						
LC05		7.85	10.4	9.94	10.1	10.1	0.21	1.28	1.44	1.29	1.17	1.30	0.13
LC08		16.3	3.78	2.90	1.20	2.63	1.3						
LC09		5.46	5.18	3.18	3.73	4.03	1.0						
LC11		1.75	0.960	0.810	1.51	1.09	0.37						
LC12		9.57	12.3	12.5	12.6	12.5	0.11						
LC14		7.15	8.18	8.12	7.39	7.90	0.44						
	Avg	7.95				Avg	8.47						
	SD	5.3				SD	8.7						
	N	6				N	7	N 1				N	1



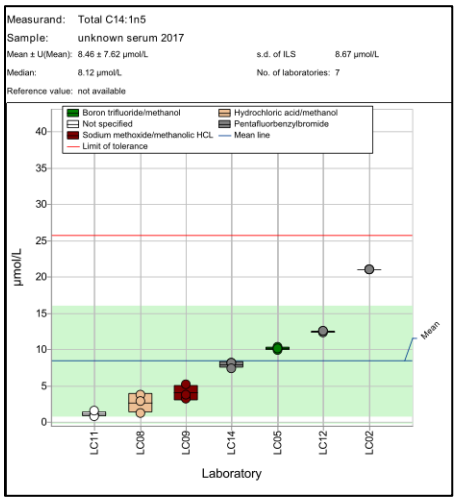
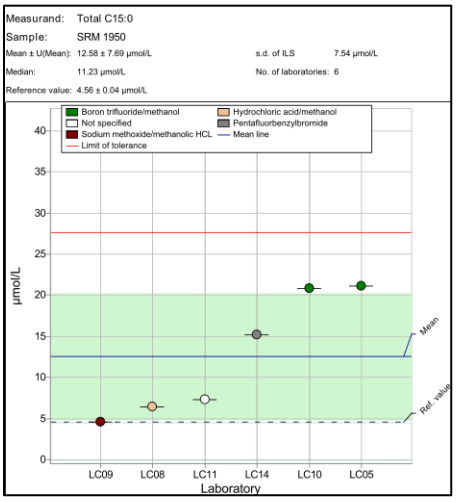


Table A-11: C15:0 Pentadecanoic acid, µmol/L

	FA		2017 Unknown, FA				FFA		2017 Unknown, FFA			
Lab	SRM 1950		A	B	C	Avg	SD	SRM 1950	A	B	C	Avg	SD
NIST	4.56 ± 0.04												
LC05		21.1	29.4	29.8	29.5	29.6	0.19						
LC08		6.47	11.5	11.7	10.6	11.3	0.56						
LC09		4.60	9.67	8.55	9.60	9.28	0.63	0.360	0.775	0.879	2.47	1.37	0.95
LC10		20.8	26.7	29.2	30.4	28.8	1.9						
LC11		7.26	12.6	21.0	20.4	18.0	4.7						
LC14		15.2	21.5	20.9	19.8	20.7	0.85						
	Avg	12.6				Avg	19.6						
	SD	7.5				SD	14						
	N	6				N	6	N 1				N	1



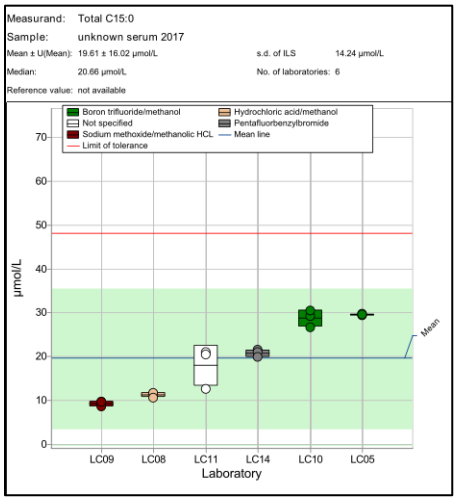
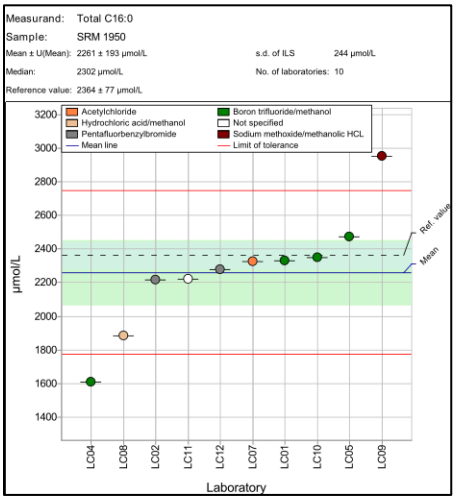
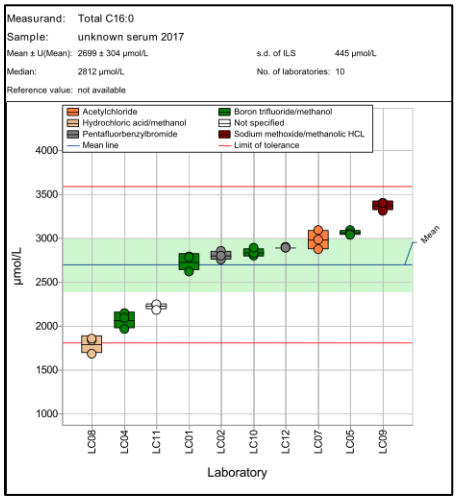
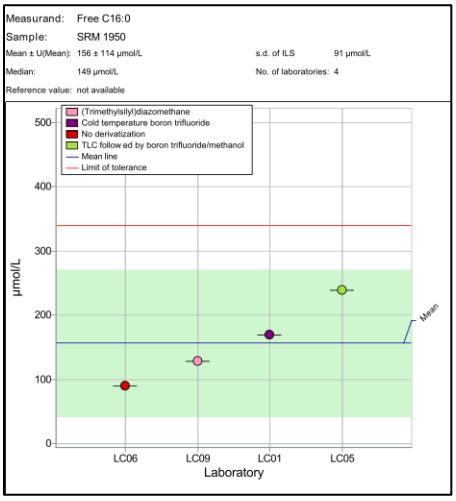


Table A-12: C16:0 Palmitic acid, µmol/L

	FA		2017 Unknown, FA					FFA		2017 Unknown, FFA			
Lab	SRM 1950		A	B	C	Avg	SD	SRM 1950		A	B	C	Avg	SD
NIST	2364 ± 77													
LC01		2332	2620	2794	2777	2730	96		169	178	180	179	179	0.86
LC02		2215	2856	2753	2800	2803	52							
LC04		1609	1967	2146	2089	2068	92							
LC05		2470	3088	3056	3039	3061	25		238	48.1	161	188	132	74
LC06									89.4	185	205	219	203	17.0
LC07		2324	3086	2871	2978	2978	108							
LC08		1882	1834	1856	1678	1790	97							
LC09		2954	3305	3398	3399	3368	54		128	176	174	180	177	3.0
LC10		2350	2800	2823	2888	2837	45							
LC11		2222	2234	2249	2186	2223	33							
LC12		2280	2890	2900	2890	2893	5.8							
	Avg	2261				Avg	2699	Avg	156				Avg	178
	SD	244				SD	445	SD	91				SD	16
	N	10				N	10	N	4				N	4







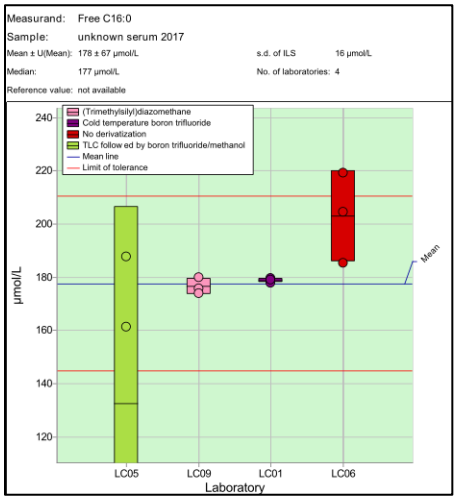
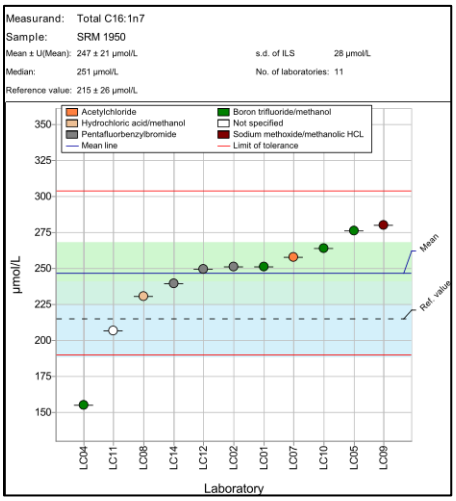
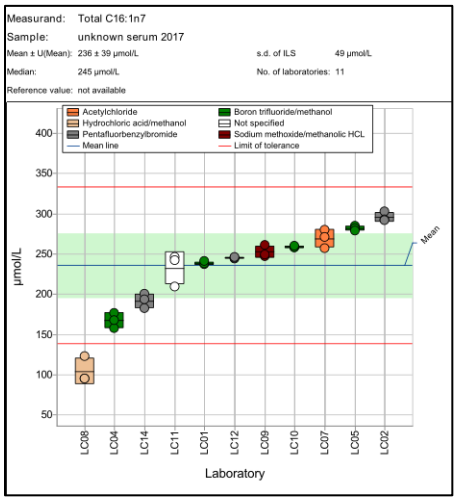
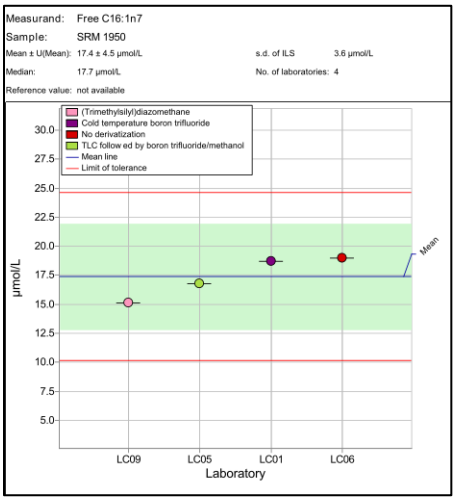


Table A-13: C16:1n7 Palmitoleic acid, µmol/L

	FA		2017 Unknown, FA				FFA		2017 Unknown, FFA			
Lab	SRM 1950		A	B	C	Avg	SD	SRM 1950	A	B	C	Avg	SD
NIST	215 ± 26												
LC01		251	237	238	241	238	2.0	18.7	12.5	13.8	13.9	13.4	0.76
LC02		251	303	293	292	296	6.1						
LC04		155	158	177	168	167	9.6						
LC05		276	285	282	279	282	3.0	16.7	15.6	15.8	16.1	15.9	0.26
LC06								19.0	9.5	13.4	17.7	13.6	4.09
LC07		258	280	257	271	269	12						
LC08		231	123	95	94	104	16						
LC09		280	247	249	261	253	7.6	15.1	14.1	13.1	12.7	13.3	0.71
LC10		264	258	258	260	259	1.4						
LC11		207	209	246	242	232	20						
LC12		249	245	245	246	245	0.70						
LC14		240	200	193	182	192	9.0						
	Avg	247				Avg	236	Avg 17.4				Avg	14.03
	SD	28				SD	49	SD 3.6				SD	2.61
	N	11				N	11	N 4				N	4







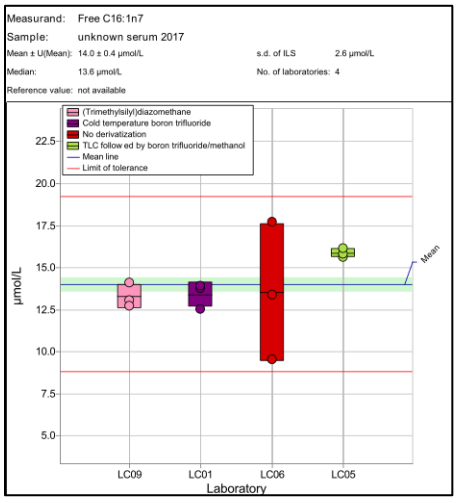
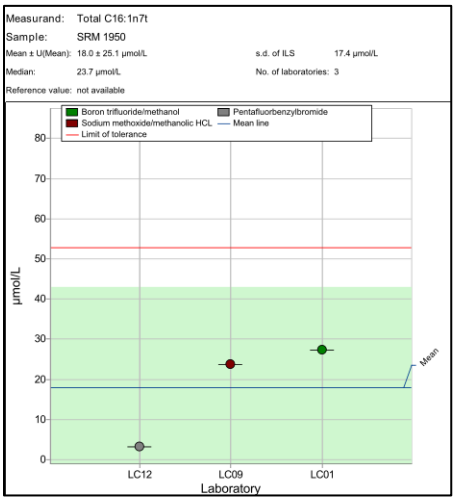
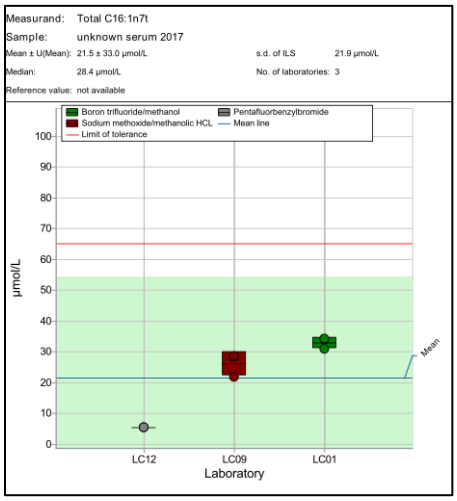
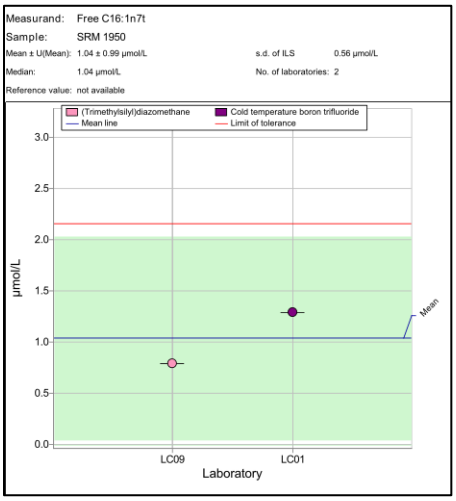


Table A-14: C16:1n7t Palmitelaidic acid, µmol/L

	FA		2017 Unknown, FA					FFA		2017 Unknown, FFA			
Lab	SRM 1950		A	B	C	Avg	SD	SRM 1950		A	B	C	Avg	SD
LC01		27.3	30.8	33.9	34.2	33.0	1.9		1.29	0.439	1.51	0.944	0.964	0.54
LC09		23.7	28.4	21.7	28.5	26.2	3.9		0.785	1.58	1.42	3.46	2.15	1.1
LC12		3.15	5.45	5.44	5.37	5.42	0.041							
LC14														
	Avg	18.0				Avg	21.5	Avg	1.04				Avg	1.56
	SD	17				SD	22	SD	0.56				SD	0.86
	N	3				N	3	N	2				N	2







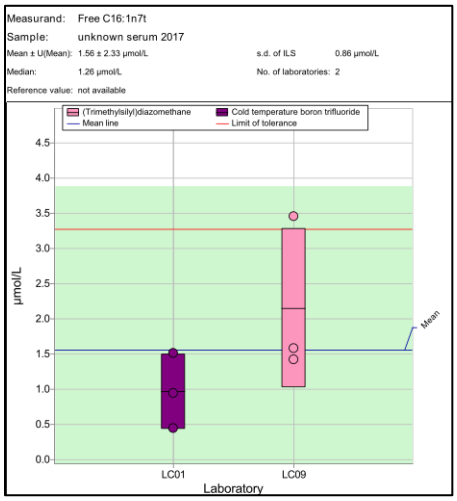
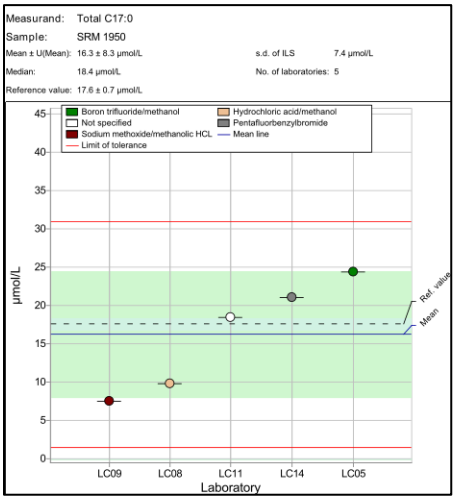
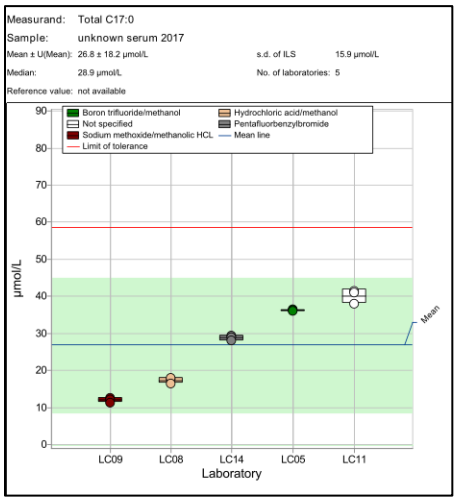
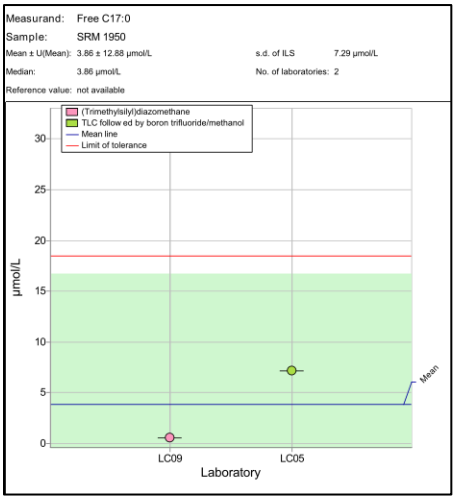


Table A-15: C17:0 Margaric acid, µmol/L

	FA		2017 Unknown, FA				FFA		2017 Unknown, FFA			
Lab	SRM 1950		A	B	C	Avg	SD	SRM 1950	A	B	C	Avg	SD
NIST	17.6 ± 0.7												
LC05		24.4	36.3	36.1	35.9	36.1	0.24	7.15	3.00	2.89	4.96	3.62	1.2
LC08		9.85	17.5	18.0	16.4	17.3	0.80						
LC09		7.52	12.6	12.0	11.3	12.0	0.65	0.579	1.09	1.22	1.25	1.19	0.087
LC11		18.4	37.9	41.4	40.9	40.1	1.9						
LC14		21.1	29.4	28.9	28.0	28.8	0.71						
	Avg	16.3				Avg	26.8	Avg 3.86				Avg	2.40
	SD	7.4				SD	16	SD 7.3				SD	3.8
	N	5				N	5	N 2				N	2







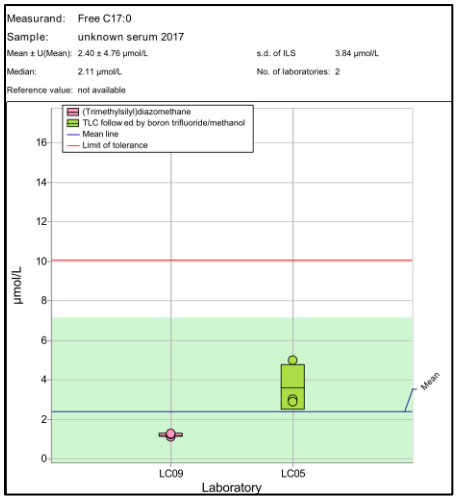
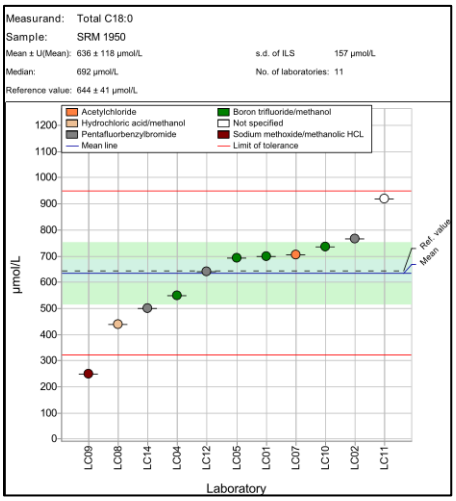
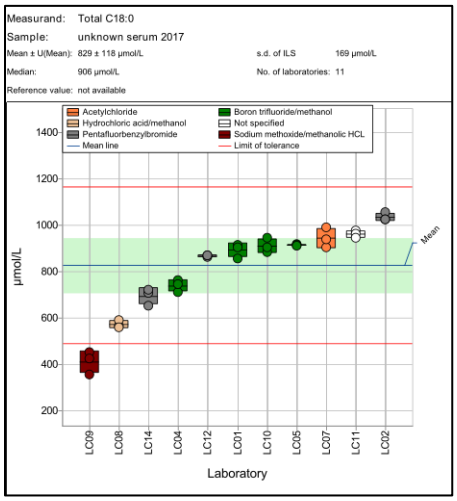
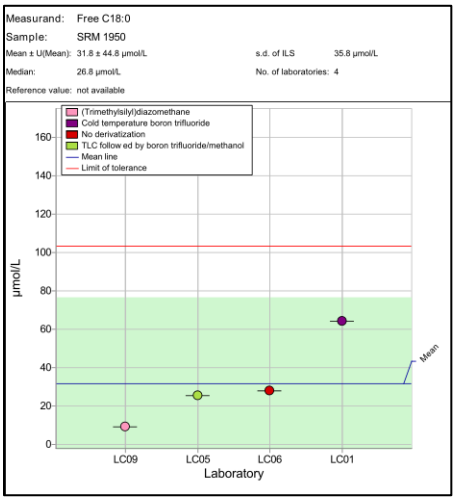


Table A-16: C18:0 Stearic acid, µmol/L

	FA		2017 Unknown, FA					FFA		2017 Unknown, FFA				
Lab	SRM 1950		A	B	C	Avg	SD	SRM 1950		A	B	C	Avg	SD
NIST	644 ± 41													
LC01		698	858	915	906	893	31		64.1	70.3	70.0	70.0	70.1	0.17
LC02		767	1055	1025	1026	1035	17							
LC04		548	713	762	745	740	25							
LC05		692	920	914	911	915	4.2		25.5	17.5	24.5	26.5	22.9	4.7
LC06									28.1	60.6	54.4	48.6	54.5	6.0
LC07		704	990	904	939	944	43							
LC08		440	566	592	559	572	17							
LC09		249	452	356	425	411	49		9.35	36.2	33.1	34.2	34.5	1.55
LC10		736	884	906	945	912	30							
LC11		919	977	963	947	963	15							
LC12		639	863	871	870	868	4.5							
LC14		499	709	723	653	695	36.8							
	Avg	636				Avg	829	Avg	31.8				Avg	45.5
	SD	157				SD	169	SD	36				SD	34
	N	11				N	11	N	4				N	4







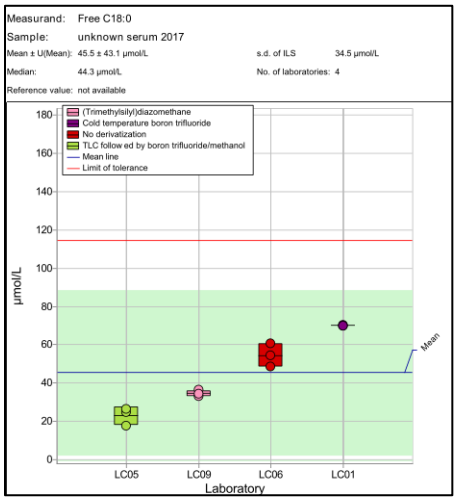
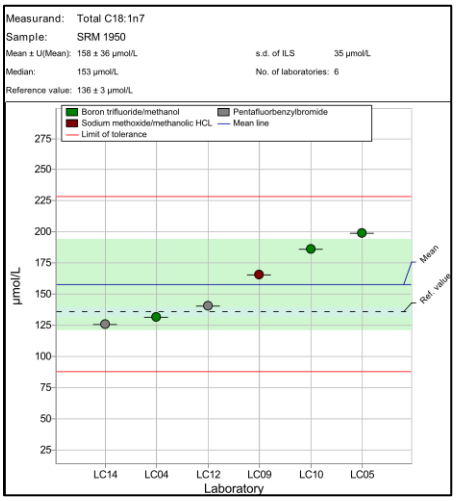
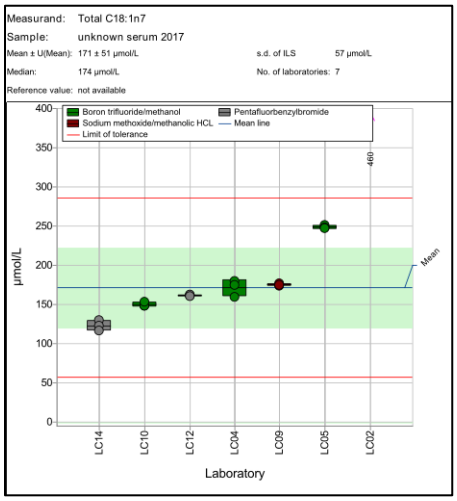
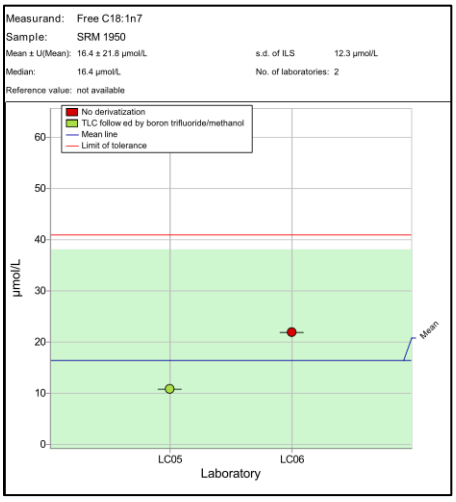


Table A-17: C18:1n7 *cis*-Vaccenic acid, µmol/L

	FA		2017 Unknown, FA					FFA		2017 Unknown, FFA				
Lab	SRM 1950		A	B	C	Avg	SD	SRM 1950		A	B	C	Avg	SD
NIST	136 ± 3													
LC02			456	484	441	460	22							
LC04		131	159	179	175	171	11							
LC05		199	251	248	247	249	2.3		10.8	11.3	11.2	11.1	11.2	0.076
LC06									21.9	16.4	17.5	17.3	17.1	0.57
LC09		166	174	177	174	175	1.6			0.306	2.64	2.92	1.95	1.4
LC10		186	148	148	153	150	2.8							
LC12		140	161	162	161	161	0.79							
LC14		126	129	122	116	123	6.6							
	Avg	158				Avg	171	Avg	16.4				Avg	10.1
	SD	35				SD	57	SD	12				SD	14
	N	6				N	7	N	2				N	3







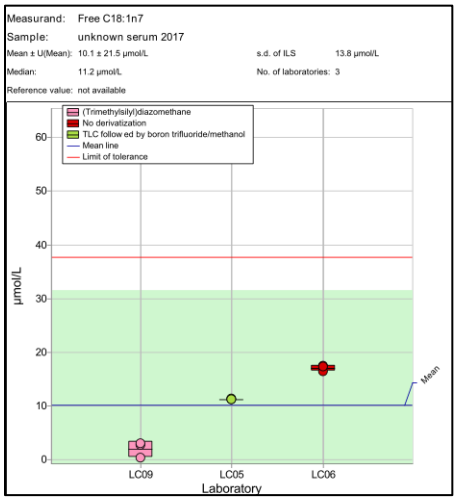
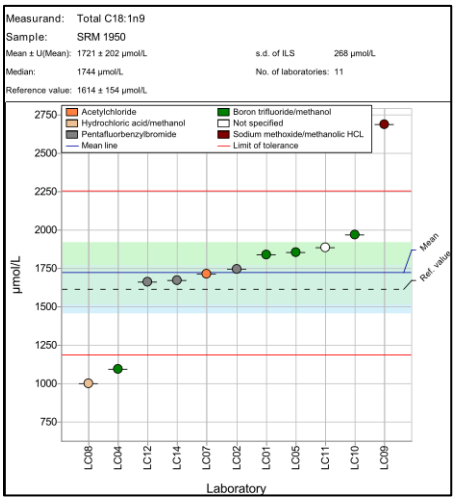
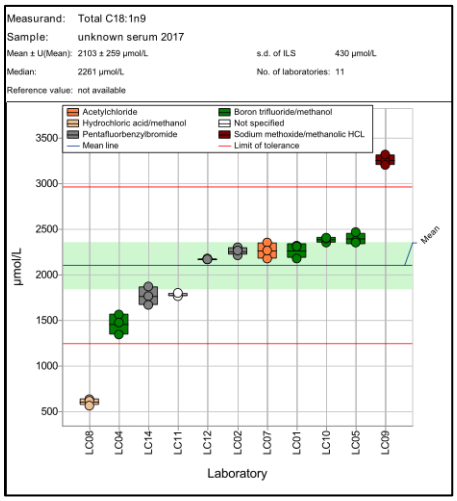
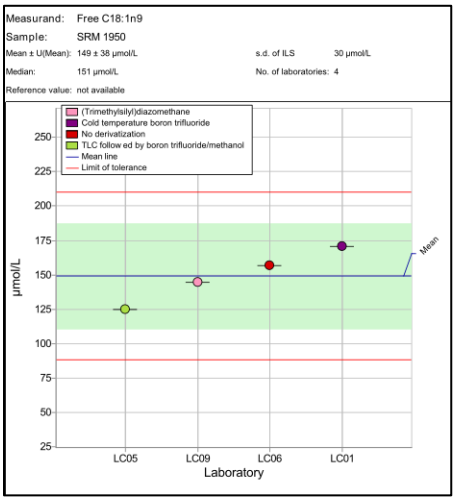


Table A-18: C18:1n9 Oleic acid, µmol/L

	FA	2017 Unknown, FA					FFA	2017 Unknown, FFA				
Lab	SRM 1950	A	B	C	Avg	SD	SRM 1950	A	B	C	Avg	SD
NIST	1614 ± 154											
LC01	1840	2173	2316	2300	2263	79	171	139	144	139	141	2.8
LC02	1744	2295	2211	2262	2256	42						
LC04	1090	1338	1560	1467	1455	112						
LC05	1852	2459	2364	2346	2390	61	125	119	120	120	120	0.44
LC06							157	114	136	127	126	11.1
LC07	1713	2348	2176	2261	2262	86						
LC08	998	631	610	560	601	37						
LC09	2688	3253	3310	3196	3253	57	144	152	149	140	147	6.3
LC10	1971	2348	2390	2405	2381	29						
LC11	1884	1769	1764	1795	1776	17						
LC12	1660	2160	2170	2160	2163	5.8						
LC14	1670	1868	1759	1665	1764	102						
	Avg 1721				Avg 2103		Avg 149				Avg 133	
	SD 268				SD 430		SD 30				SD 18	
	N 11				N 11		N 4				N 4	







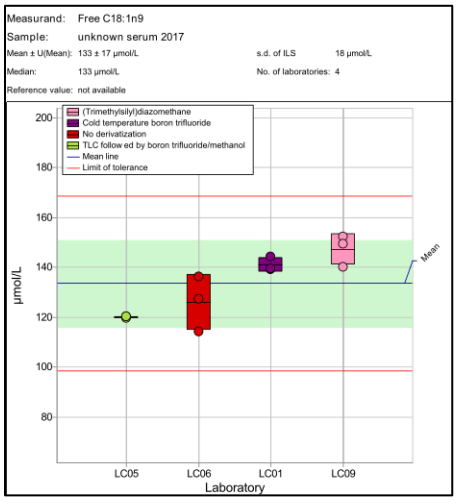
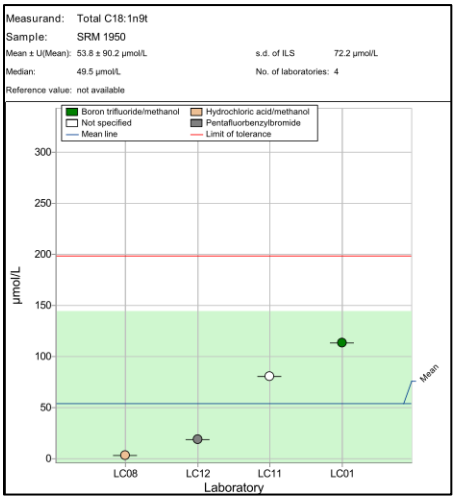


Table A-19: C18:1n9t Elaidic acid, µmol/L

	FA		2017 Unknown, FA					FFA		2017 Unknown, FFA				
Lab	SRM 1950		A	B	C	Avg	SD	SRM 1950		A	B	C	Avg	SD
LC01		113	156	171	182	170	13.0		20.1	15.0	20.4	16.2	17.2	2.8
LC08		3.15	0.500	2.12	0.450	1.02	0.95							
LC11		80.5	108	102	110	107	4.3							
LC12		18.5	30.8	30.8	31.0	30.9	0.10							
	Avg	53.8				Avg	77.0							
	SD	72				SD	140							
	N	4				N	4	N	1				N	1



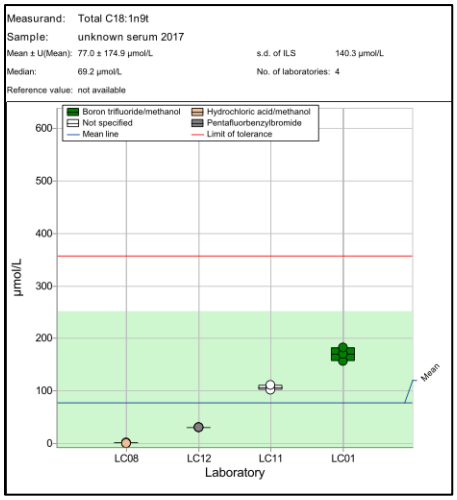
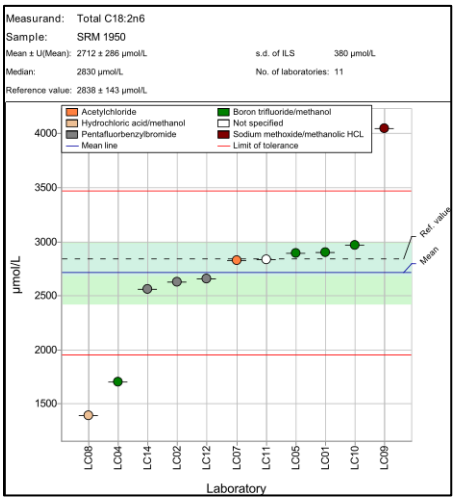
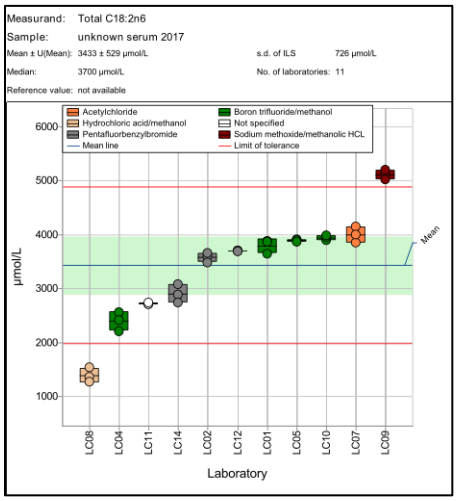
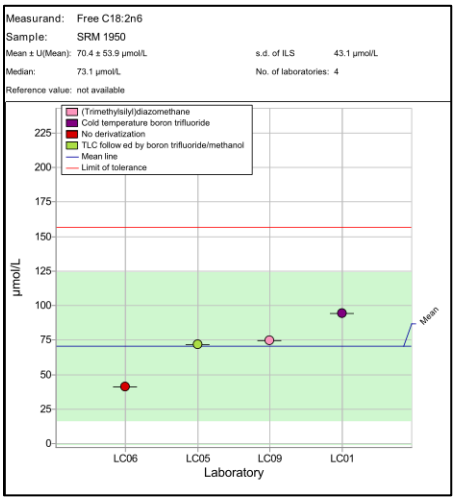


Table A-20: C18:2n6 Linoleic acid, µmol/L

	FA	2017 Unknown, FA					FFA	2017 Unknown, FFA				
Lab	SRM 1950	A	B	C	Avg	SD	SRM 1950	A	B	C	Avg	SD
NIST	2838 ± 143											
LC01	2903	3639	3879	3858	3792	133	94.1	113	113	113	113	0.082
LC02	2629	3590	3482	3651	3574	86						
LC04	1704	2209	2561	2403	2391	176						
LC05	2896	3913	3888	3859	3886	27	71.6	92.8	92.5	92.7	92.7	0.16
LC06							41.3	108	150	124	128	21
LC07	2830	4143	3842	3997	3994	151						
LC08	1393	1529	1352	1267	1383	134						
LC09	4049	5115	5196	5023	5111	86	74.6	107	104	94.6	102	6.5
LC10	2970	3885	3950	3980	3938	48						
LC11	2835	2714	2713	2734	2720	12						
LC12	2660	3700	3700	3690	3697	5.8						
LC14	2562	3082	2888	2741	2904	171						
	Avg 2712				Avg 3433		Avg 70.4				Avg 109	
	SD 380				SD 726		SD 43				SD 20	
	N 11				N 11		N 4				N 4	







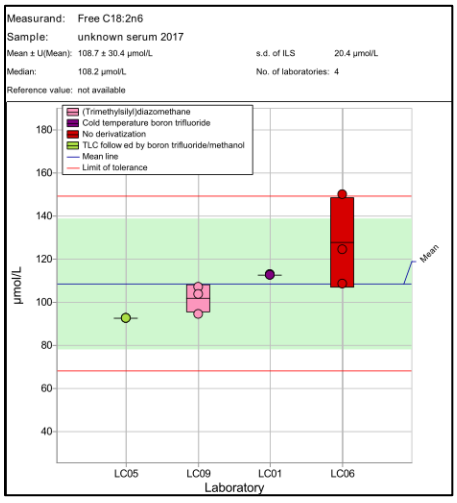
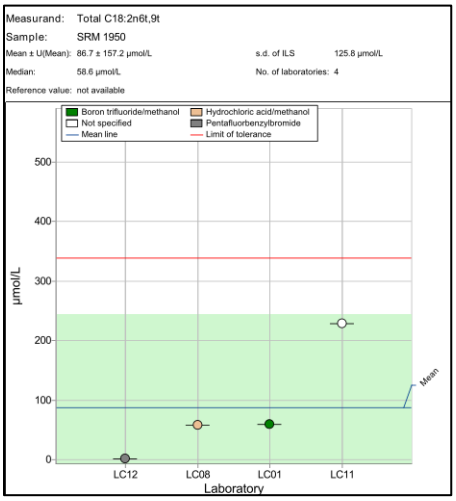


Table A-21: C18:2n6t,9t Linoelaidic acid, µmol/L

	FA	2017 Unknown, FA					FFA	2017 Unknown, FFA				
Lab	SRM 1950	A	B	C	Avg	SD	SRM 1950	A	B	C	Avg	SD
LC01	59.0	88.6	77.0	81.4	82.3	5.9	8.95	9.71	8.97	5.74	8.14	2.1
LC08	58.3	55.4	56.4	57.5	56.4	1.0						
LC10	<LOD	20.8	20.3	<LOD	20.5	0.30						
LC11	228	222	230	234	229	6.3						
LC12	1.60	2.08	1.88	2.09	2.01	0.12						
	Avg 86.7				Avg 70.2							
	SD 126				SD 80							
	N 4				N 5		N 1				N 1	



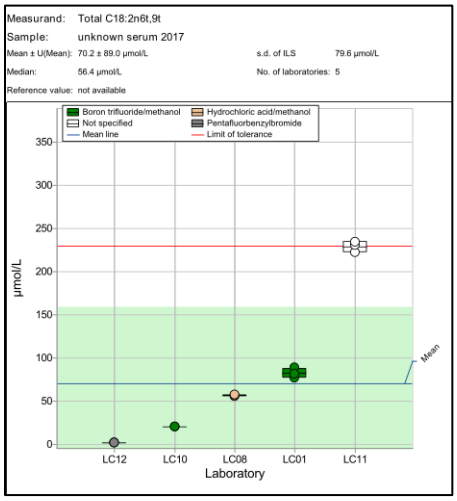
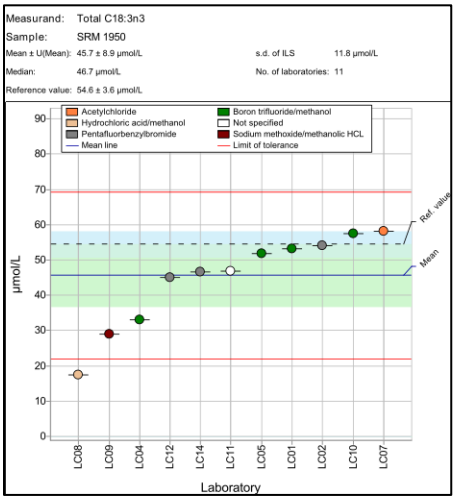
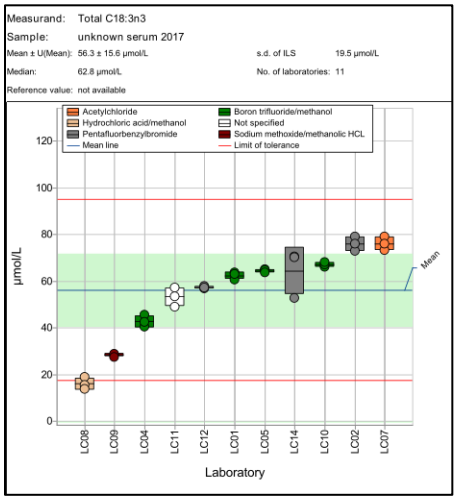
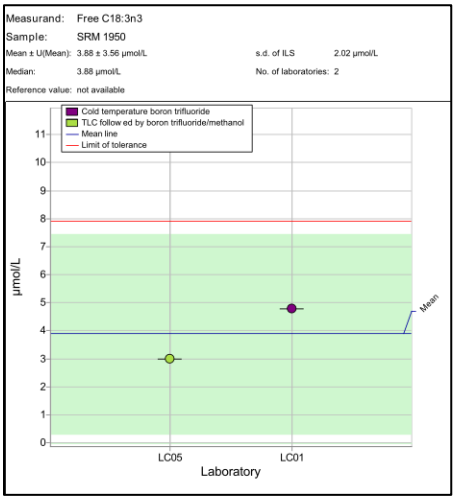


Table A-22: C18:3n3 α-Linolenic acid, µmol/L

	FA		2017 Unknown, FA				FFA		2017 Unknown, FFA			
Lab	SRM 1950		A	B	C	Avg	SD	SRM 1950		A	B	C	Avg	SD
NIST	54.6 ± 3.6													
LC01		53.1	60.6	63.6	62.8	62.3	1.6		4.79	3.72	3.71	4.58	4.00	0.50
LC02		54.0	79.0	73.0	76.0	76.0	3.0							
LC04		33.0	40.2	45.4	42.4	42.7	2.6							
LC05		51.8	64.9	64.7	63.7	64.4	0.65		2.97	2.93	2.90	2.72	2.85	0.11
LC07		58.2	79.0	73.3	75.8	76.0	2.9							
LC08		17.4	18.9	15.4	13.9	16.0	2.6							
LC09		28.9	28.5	28.9	27.5	28.3	0.71							
LC10		57.4	66.0	67.1	68.1	67.1	1.1							
LC11		46.7	49.0	53.5	57.1	53.2	4.0							
LC12		45.0	56.8	57.9	57.2	57.3	0.56							
LC14		46.5	70.3	70.2	52.8	64.5	10.1							
	Avg	45.7				Avg	56.3	Avg	3.88				Avg	3.43
	SD	12				SD	20	SD	2.0				SD	1.8
	N	11				N	11	N	2				N	2







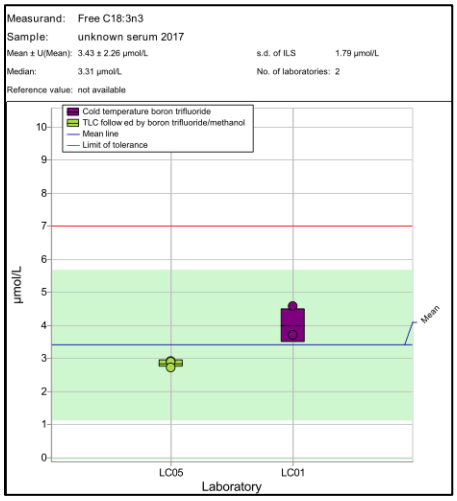
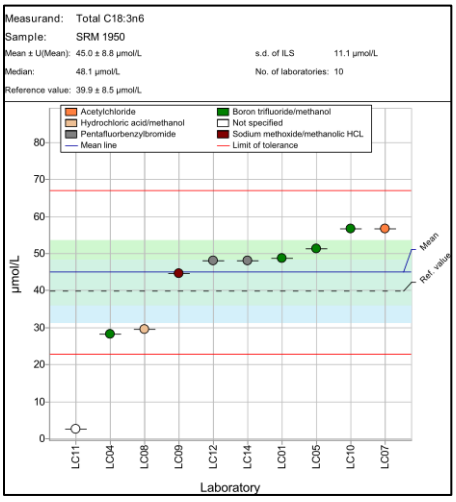
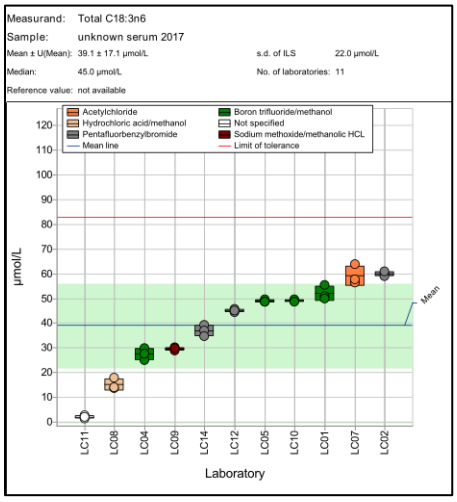
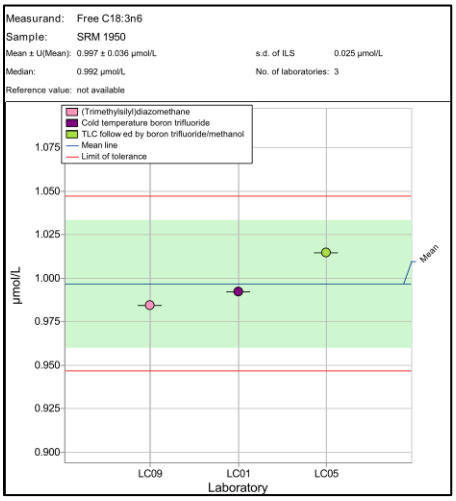


Table A-23: C18:3n6 γ-Linolenic acid, µmol/L

	FA	2017 Unknown, FA					FFA	2017 Unknown, FFA				
Lab	SRM 1950	A	B	C	Avg	SD	SRM 1950	A	B	C	Avg	SD
NIST	39.9 ± 8.5											
LC01	48.7	50.7	55.5	49.9	52.0	3.0	0.992	0.651	0.672	0.583	0.635	0.047
LC02		60.0	59.0	61.0	60.0	1.0						
LC04	28.2	24.9	29.8	27.6	27.5	2.4						
LC05	51.3	49.6	48.6	48.5	48.9	0.62	1.02	1.09	1.14	0.900	1.04	0.13
LC07	56.7	63.9	56.4	57.5	59.3	4.1						
LC08	29.5	17.9	13.7	13.8	15.1	2.4						
LC09	44.5	28.9	30.2	29.7	29.6	0.66	0.984	0.764	0.838	1.13	0.911	0.19
LC10	56.6	49.0	49.6	48.6	49.1	0.50						
LC11	2.50	2.46	1.23	2.08	1.92	0.63						
LC12	48.1	44.4	45.6	45.0	45.0	0.61						
LC14	48.2	39.3	36.9	34.7	37.0	2						
	Avg 45.0				Avg 39.1		Avg 0.997				Avg 0.863	
	SD 11				SD 22		SD 0.025				SD 0.38	
	N 10				N 11		N 3				N 3	







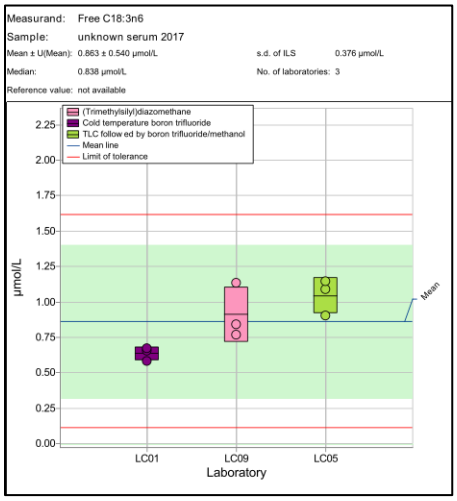
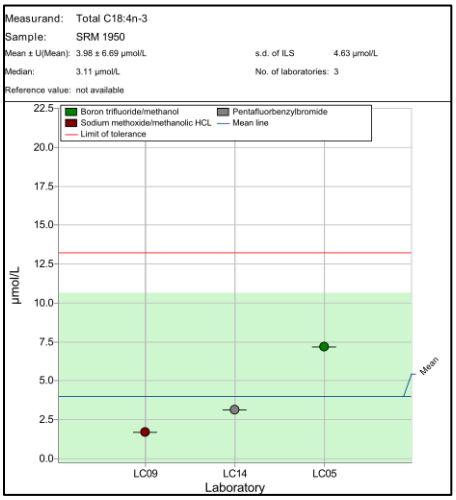
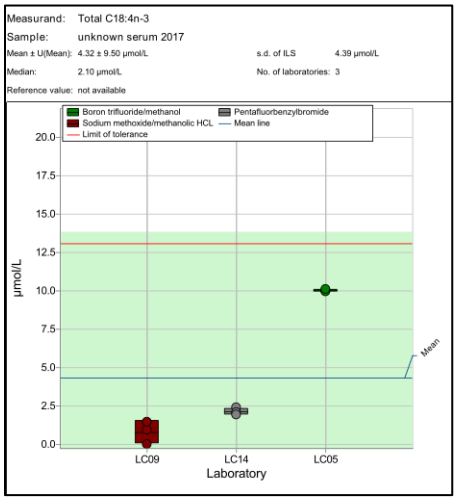
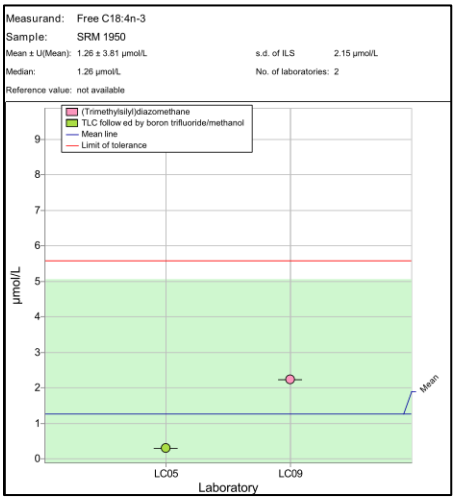


Table A-24: C18:4n3 Stearidonic acid, µmol/L

	FA		2017 Unknown, FA					FFA		2017 Unknown, FFA				
Lab	SRM 1950		A	B	C	Avg	SD	SRM 1950		A	B	C	Avg	SD
LC05		7.16	9.97	9.96	10.1	10.0	0.068		0.291	0.303	0.296	0.219	0.273	0.047
LC09		1.68	0.00	0.95	1.47	0.805	0.74		2.23	2.20	2.14	2.20	2.18	0.038
LC14		3.11	2.38	2.10	1.94	2.14	0.23							
	Avg	3.98				Avg	4.32	Avg	1.26				Avg	1.23
	SD	4.6				SD	4.4	SD	2.2				SD	4.2
	N	3				N	3	N	2				N	2







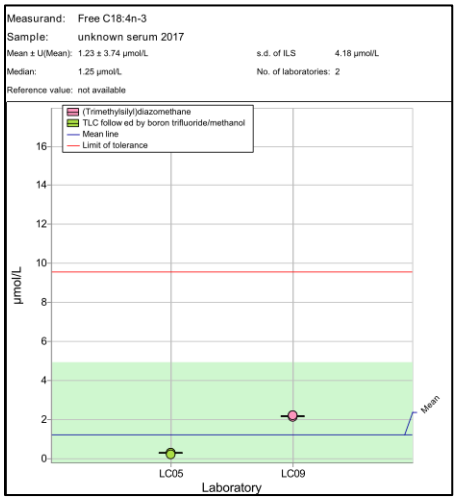
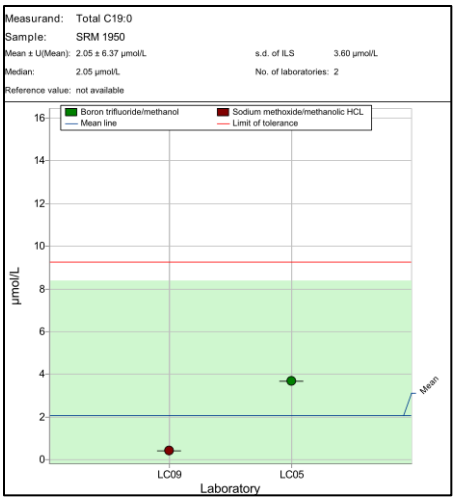


Table A-25: C19:0 Nonadecanoic acid, µmol/L

	FA		2017 Unknown, FA				FFA		2017 Unknown, FFA			
Lab	SRM 1950		A	B	C	Avg	SD	SRM 1950	A	B	C	Avg	SD
LC05		3.67	5.43	4.72	4.74	4.96	0.40	0.387	0.404	0.450	0.242	0.365	0.11
LC09		0.423	0.659	0.671	0.825	0.718	0.093						
	Avg	2.05				Avg	2.84						
	SD	3.6				SD	8.9						
	N	2				N	2	N 1				N	1



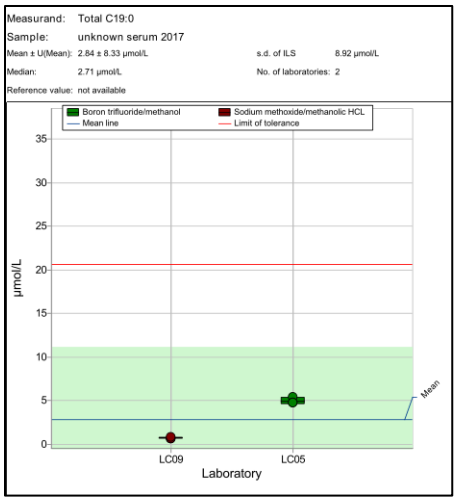
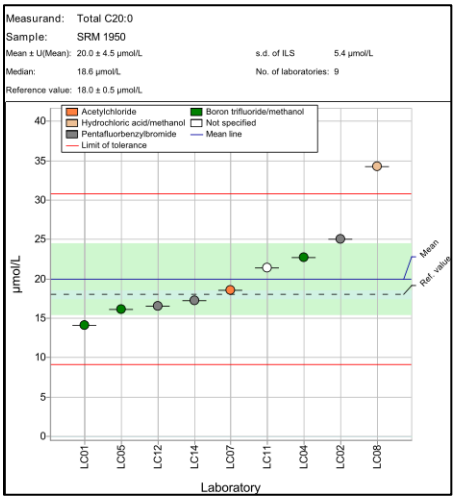
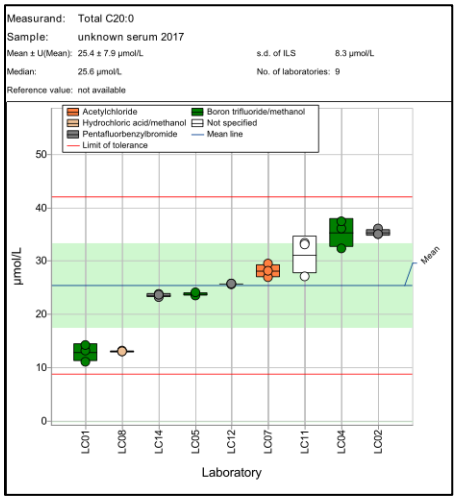
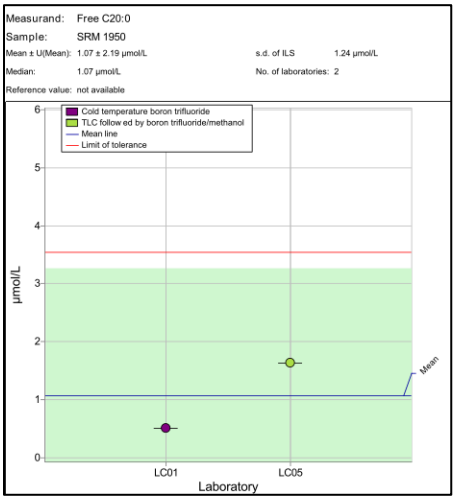


Table A-26: C20:0 Arachidic acid, µmol/L

	FA		2017 Unknown, FA				FFA		2017 Unknown, FFA			
Lab	SRM 1950		A	B	C	Avg	SD	SRM 1950	A	B	C	Avg	SD
NIST	18.0 ± 0.5												
LC01	14.14		11.0	13.2	14.3	12.8	1.6	0.512	0.395	0.324	0.397	0.372	0.042
LC02	25.0		36.0	35.0	35.0	35.3	0.58						
LC04	22.7		32.2	36.1	37.4	35.3	2.7						
LC05	16.1		23.8	23.5	24.1	23.8	0.30	1.63	1.43	1.53	1.56	1.51	0.071
LC07	18.6		29.4	26.9	28.1	28.1	1.3						
LC08	34.3		13.2	13.0	13.0	13.0	0.12						
LC11	21.4		27.1	33.4	33.1	31.2	3.6						
LC12	16.5		25.7	25.6	25.6	25.6	0.087						
LC14	17.2		23.8	23.2	23.7	23.5	0.32						
	Avg	20.0				Avg	25.4	Avg	1.07			Avg	0.939
	SD	5.4				SD	8.3	SD	1.2			SD	2.4
	N	9				N	9	N	2			N	2







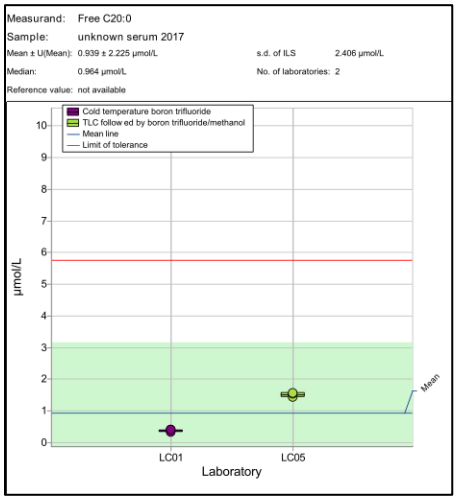
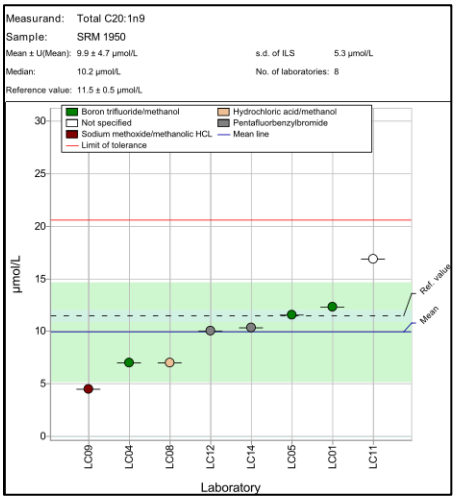
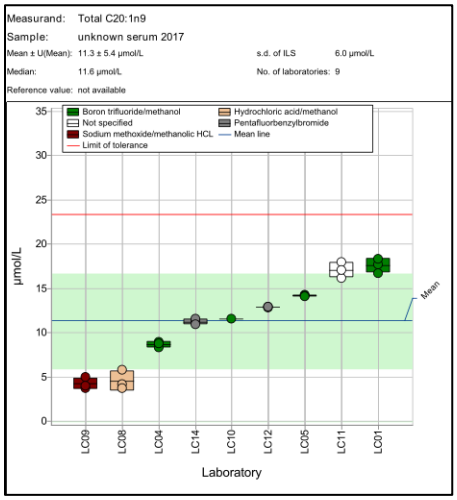
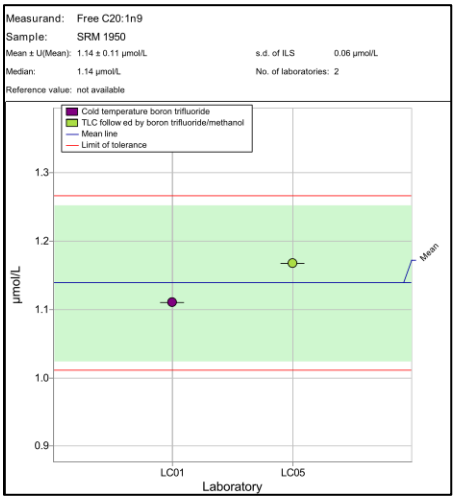


Table A-27: C20:1n9 11-Eicosenoic acid, µmol/L

	FA		2017 Unknown, FA					FFA		2017 Unknown, FFA			
Lab	SRM 1950		A	B	C	Avg	SD	SRM 1950		A	B	C	Avg	SD
NIST	11.5 ± 0.5													
LC01	12.4		17.7	16.7	18.3	17.6	0.82		1.11	0.856	0.655	0.730	0.747	0.10
LC04	6.98		8.26	8.96	8.77	8.67	0.36							
LC05	11.5		14.3	14.2	14.1	14.2	0.10		1.17	1.12	1.37	1.13	1.21	0.14
LC08	6.99		5.80	4.12	3.72	4.55	1.1							
LC09	4.48		3.74	3.99	4.94	4.22	0.63							
LC10	<LOD		11.6	<LOD	<LOD	11.6								
LC11	16.8		17.9	16.2	17.1	17.1	0.86							
LC12	10.1		12.8	12.9	12.9	12.9	0.054							
LC14	10.3		11.3	11.5	10.9	11.2	0.32							
	Avg	9.9				Avg	11.3	Avg	1.14				Avg	0.978
	SD	5.3				SD	6.0	SD	0.064				SD	0.81
	N	8				N	9	N	2				N	2







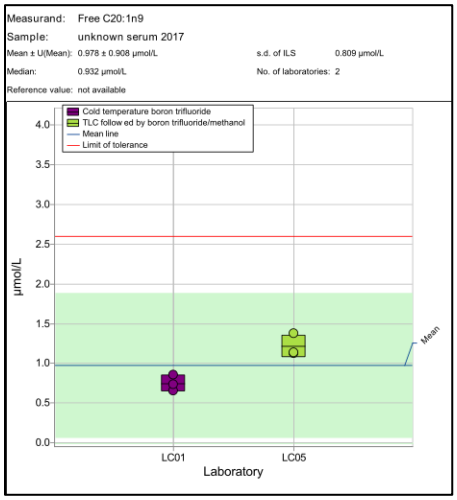
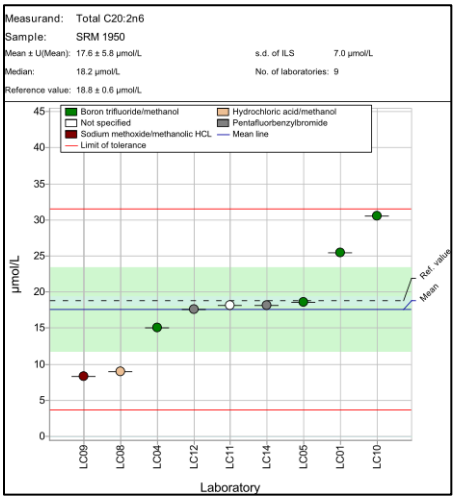
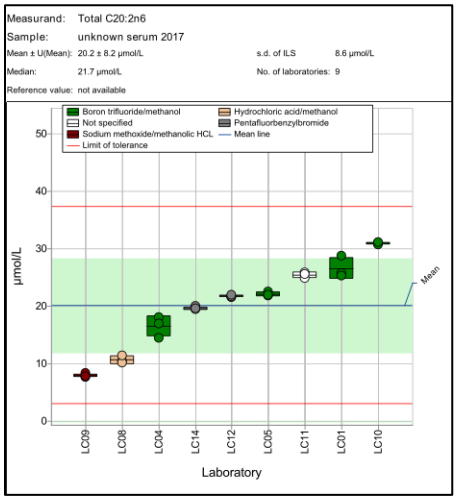
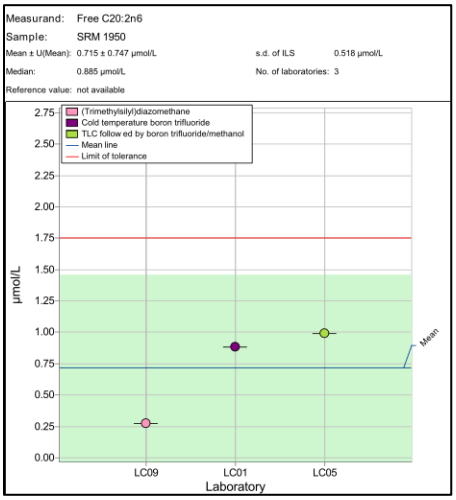


Table A-28: C20:2n6 11,14-Eicosadienoic acid, µmol/L

	FA		2017 Unknown, FA				FFA		2017 Unknown, FFA			
Lab	SRM 1950		A	B	C	Avg	SD	SRM 1950	A	B	C	Avg	SD
NIST	18.8 ± 0.6												
LC01		25.5	28.7	25.7	25.3	26.6	1.9	0.885	0.834	0.651	0.636	0.707	0.11
LC04		15.0	14.5	18.0	17.0	16.5	1.8						
LC05		18.6	21.8	22.5	22.0	22.1	0.37	0.991	0.855	0.925	0.842	0.874	0.045
LC08		8.96	10.4	10.1	11.5	10.6	0.72						
LC09		8.27	7.69	8.28	7.75	7.91	0.33	0.270	0.234	0.312	0.247	0.264	0.042
LC10		30.6	30.9	30.7	31.1	30.9	0.19						
LC11		18.2	24.9	25.9	25.5	25.4	0.49						
LC12		17.6	21.5	21.7	22.0	21.7	0.25						
LC14		18.2	19.9	19.5	19.5	19.6	0.26						
	Avg	17.6				Avg	20.2	Avg	0.715			Avg	0.615
	SD	7.0				SD	8.6	SD	0.52			SD	0.52
	N	9				N	9	N	3			N	3







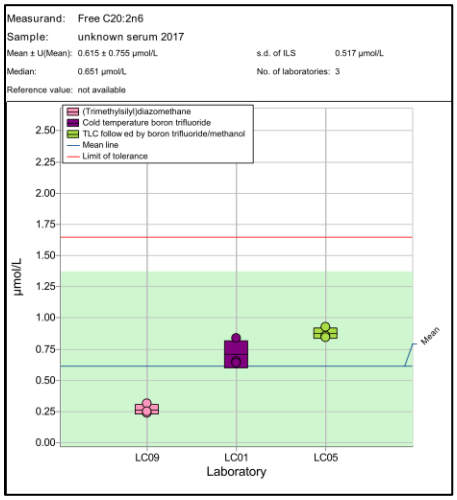
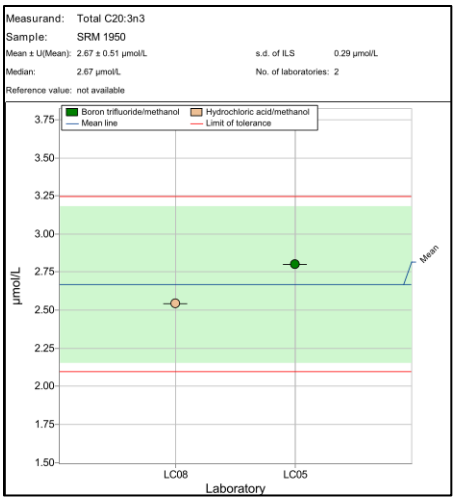


Table A-29: C20:3n3 11,14,17-Eicosatrienoic acid, µmol/L

	FA		2017 Unknown, FA					FFA	2017 Unknown, FFA				
Lab	SRM 1950		A	B	C	Avg	SD	SRM 1950	A	B	C	Avg	SD
LC05		2.80	2.30	3.04	2.90	2.75	0.40	0.406	0.350	0.402	0.375	0.376	0.026
LC08		2.54	6.68	6.39	6.12	6.40	0.28						
	Avg	2.7				Avg	4.57						
	SD	0.3				SD	7.4						
	N	2				N	2	N 1				N	1



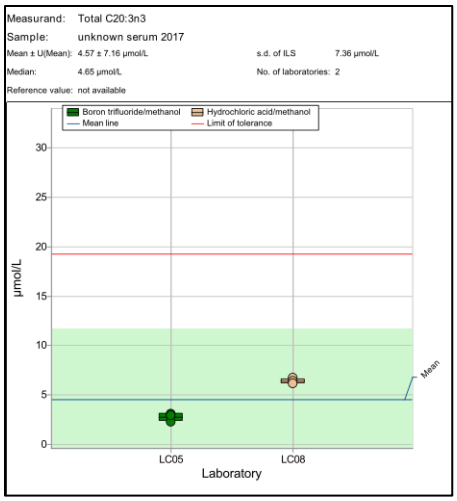
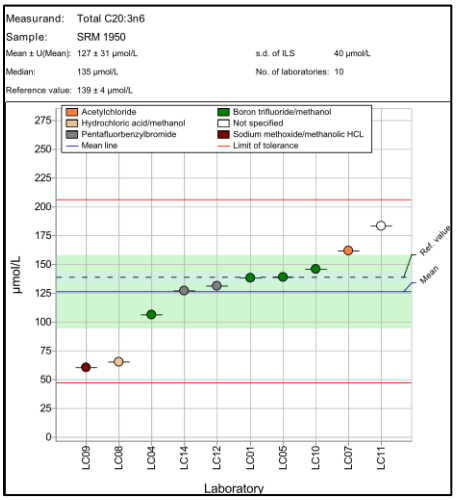
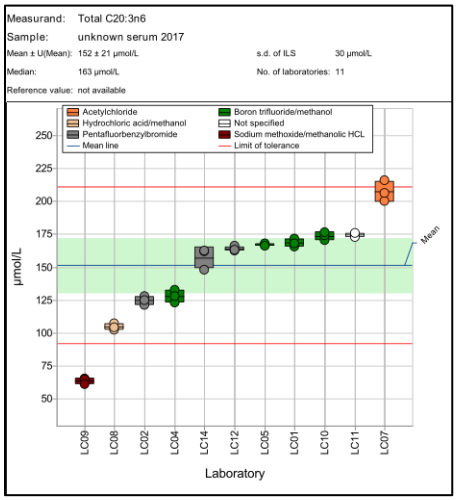
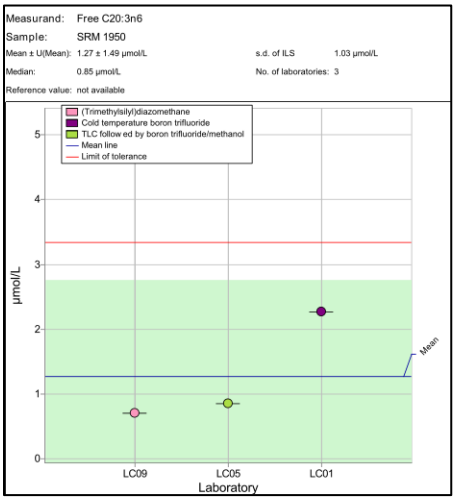


Table A-30: C20:3n6 homo-γ-Linolenic acid, µmol/L

	FA		2017 Unknown, FA					FFA	2017 Unknown, FFA				
Lab	SRM 1950		A	B	C	Avg	SD	SRM 1950	A	B	C	Avg	SD
NIST	139 ± 4												
LC01		138	166	172	168	168	3.1	2.27	3.80	3.34	4.04	3.73	0.36
LC02			128	121	125	125	3.5						
LC04		106	123	132	128	128	4.6						
LC05		139	167	168	166	167	0.90	0.848	1.87	1.81	1.76	1.81	0.056
LC07		162	216	200	206	207	8.1						
LC08		65.5	102	107	104	105	2						
LC09		60.6	65	65	61	63.9	2.38	0.700	1.12	1.23	0.855	1.07	0.19
LC10		146	170	174	176	174	3.13						
LC11		183	174	173	176	174	1.44						
LC12		131	163	166	163	164	1.73						
LC14		127	162	162	148	157	8.1						
	Avg	127				Avg	152	Avg 1.27				Avg	2.20
	SD	40				SD	30	SD 1.0				SD	2.0
	N	10				N	11	N 3				N	3







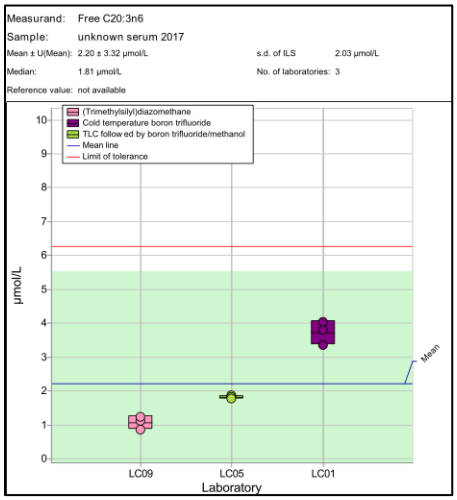
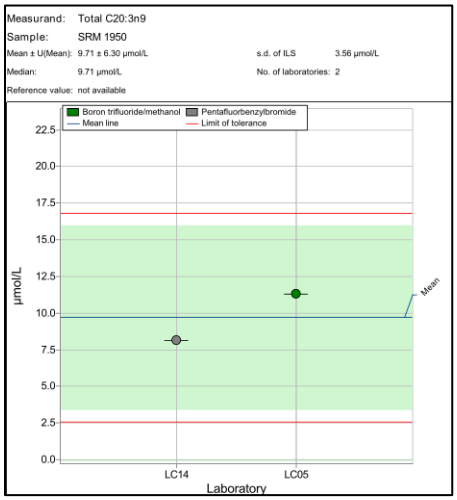


Table A-31: C20:3n9 5,8,11-Eicosatrienoic acid (Mead acid), µmol/L

	FA		2017 Unknown, FA					FFA		2017 Unknown, FFA				
Lab	SRM 1950		A	B	C	Avg	SD	SRM 1950		A	B	C	Avg	SD
LC02			19.0	18.0	19.0	18.7	0.58							
LC05		11.3	8.15	8.94	8.51	8.53	0.40		0.178	0.213	0.276	0.174	0.221	0.051
LC14		8.10	6.02	5.63	5.54	5.73	0.25							
	Avg	9.71				Avg	11.0							
	SD	3.6				SD	6.8							
	N	2				N	3	N	1				N	1



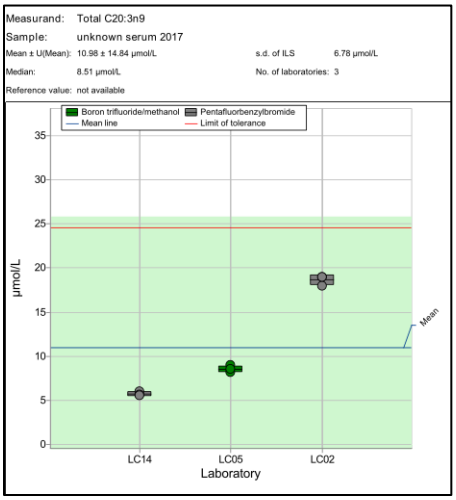
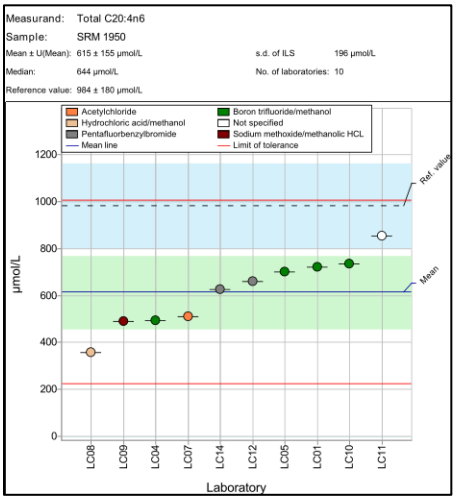
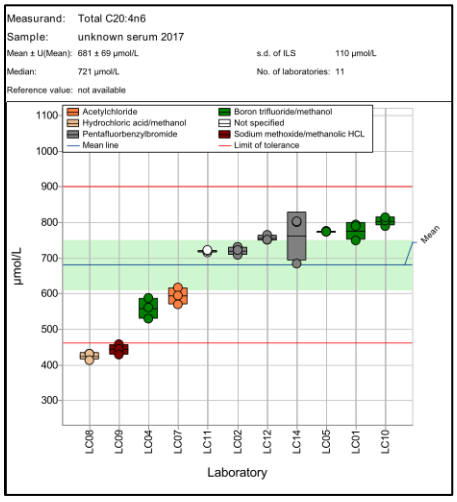
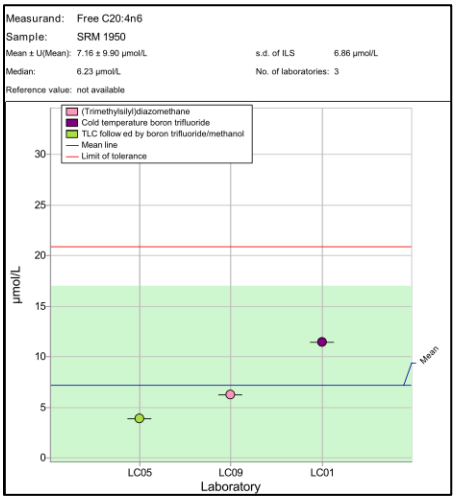


Table A-32: C20:4n6 Arachidonic acid, µmol/L

	FA	2017 Unknown, FA					FFA	2017 Unknown, FFA				
Lab	SRM 1950	A	B	C	Avg	SD	SRM 1950	A	B	C	Avg	SD
NIST	984 ± 180											
LC01	720	748	792	789	776	25	11.4	19.9	18.1	19.2	19.1	0.87
LC02		731	709	721	720	11						
LC04	495	529	587	561	559	29						
LC05	701	776	774	772	774	2.2	3.83	10.9	10.8	10.7	10.8	0.097
LC07	510	616	570	594	593	23						
LC08	358	430	431	413	425	10						
LC09	489	458	445	428	443	15	6.23	8.78	8.91	7.98	8.56	0.50
LC10	734	789	805	814	802	12						
LC11	853	715	720	722	719	3.5						
LC12	661	751	764	750	755	7.7						
LC14	627	800	801	683	761	68						
	Avg 615				Avg 681		Avg 7.16				Avg 12.8	
	SD 196				SD 110		SD 6.9				SD 6.0	
	N 10				N 11		N 3				N 3	







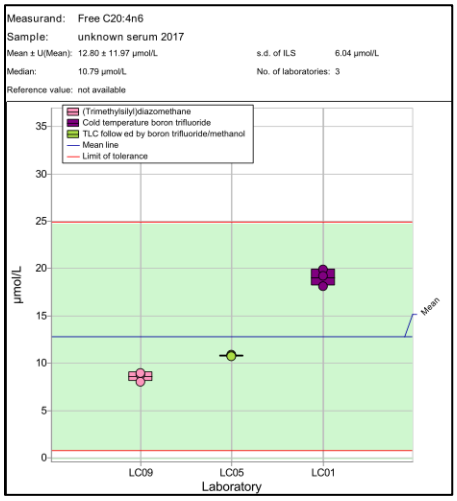
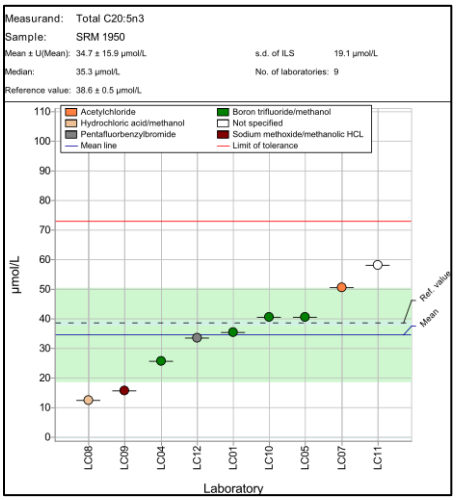
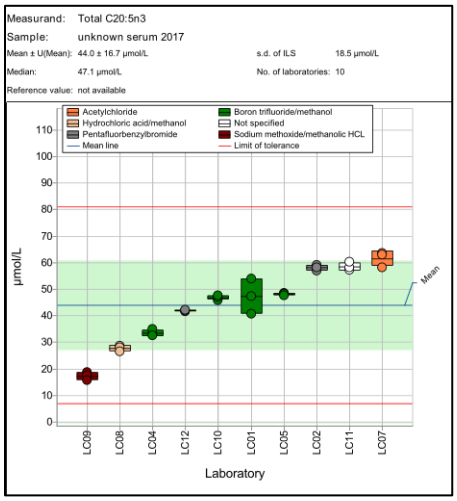
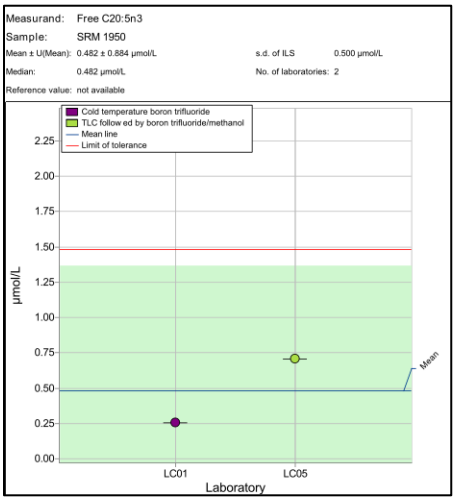


Table A-33: C20:5n3 Eicosapentaenoic acid, µmol/L

	FA		2017 Unknown, FA				FFA		2017 Unknown, FFA			
Lab	SRM 1950		A	B	C	Avg	SD	SRM 1950		A	B	C	Avg	SD
NIST	38.6 ± 0.5													
LC01		35.3	40.5	47.3	53.8	47.2	6.7		0.257	2.34	0.465	1.01	1.27	0.96
LC02			59.0	57.0	58.0	58.0	1.0							
LC04		25.8	33.1	34.9	32.5	33.5	1.2							
LC05		40.6	48.5	48.2	47.6	48.1	0.46		0.708	0.985	1.05	1.06	1.03	0.040
LC07		50.6	63.5	58.2	62.8	61.5	2.9							
LC08		12.4	28.7	27.9	26.5	27.7	1.1							
LC09		15.6	18.7	17.5	15.7	17.3	1.5			0.470	0.534	0.343	0.449	0.10
LC10		40.6	45.9	47.0	47.4	46.8	0.80							
LC11		58.0	57.1	58.1	60.2	58.4	1.6							
LC12		33.4	41.4	41.8	42.1	41.8	0.37							
	Avg	34.7				Avg	44	Avg	0.482				Avg	0.917
	SD	19				SD	19	SD	0.50				SD	1.0
	N	9				N	10	N	2				N	3







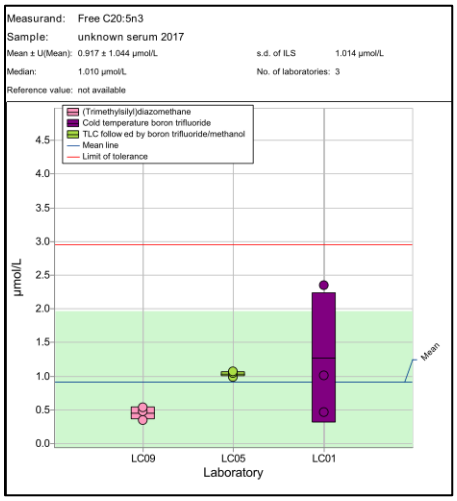
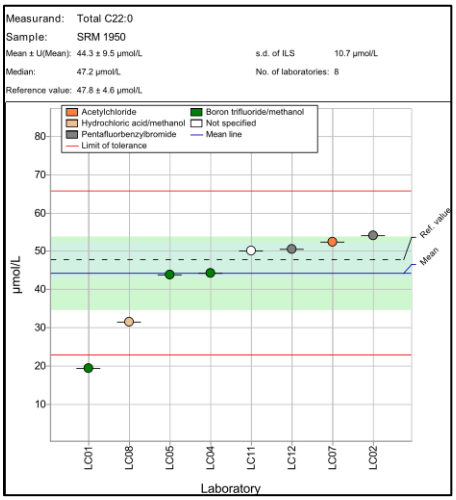
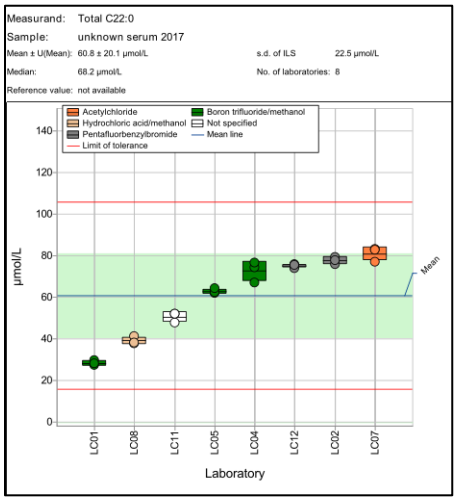
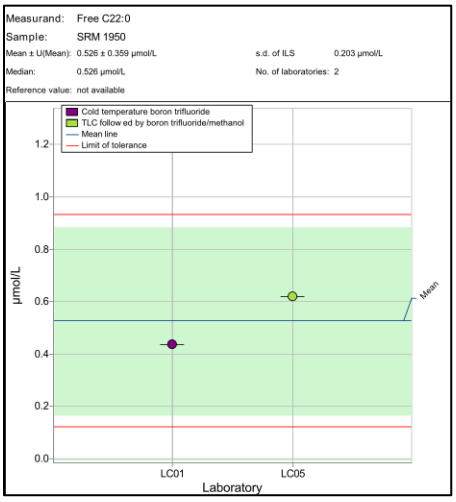


Table A-34: C22:0 Docosanoic acid, µmol/L

	FA	2017 Unknown, FA					FFA	2017 Unknown, FFA				
Lab	SRM 1950	A	B	C	Avg	SD	SRM 1950	A	B	C	Avg	SD
NIST	47.8 ± 4.6											
LC01	19.4	27.2	29.6	27.9	28.2	1.3	0.435	0.684	0.317	0.293	0.431	0.22
LC02	54.0	79.3	75.7	77.7	77.6	1.8						
LC04	44.3	67.0	74.2	76.3	72.5	4.9						
LC05	43.8	62.0	62.2	64.1	62.8	1.2	0.618	0.567	0.531	0.571	0.556	0.022
LC07	52.3	83.1	77.0	82.5	80.9	3.4						
LC08	31.5	41.2	37.8	38.1	39.0	1.9						
LC11	50.1	52.0	47.5	52.0	50.5	2.6						
LC12	50.4	73.9	75.6	75.4	75.0	0.90						
	Avg 44.3				Avg 60.8		Avg 0.526				Avg 0.494	
	SD 11				SD 22		SD 0.20				SD 0.32	
	N 8				N 8		N 2				N 2	







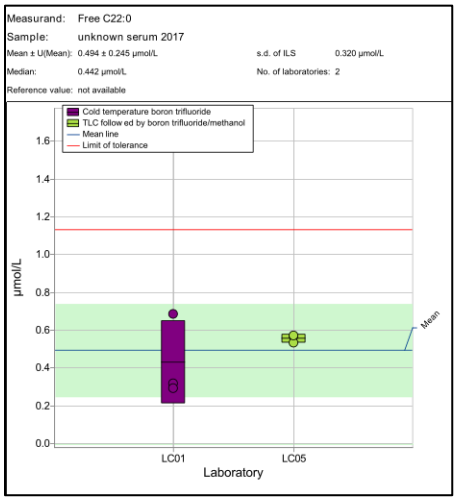
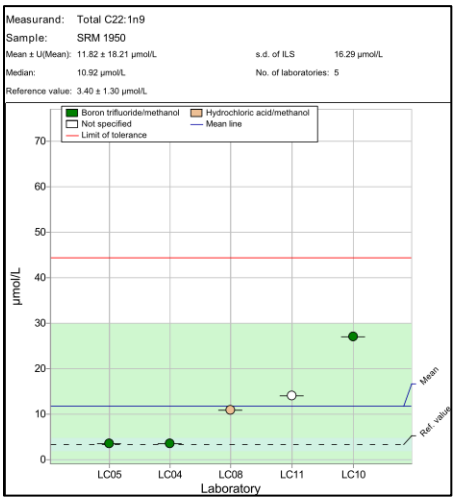


Table A-35: C22:1n9 Docosenoic acid, µmol/L

	FA	2017 Unknown, FA					FFA	2017 Unknown, FFA				
Lab	SRM 1950	A	B	C	Avg	SD	SRM 1950	A	B	C	Avg	SD
LC02		10.0	10.0	10.0	10.0	0.0						
LC04	3.58	4.37	3.25	2.53	3.38	0.92						
LC05	3.43	3.95	3.51	4.60	4.02	0.55	0.984	1.32	1.20	1.35	1.29	0.078
LC08	10.9	0.960	0.580	0.360	0.633	0.30						
LC10	27.1	12.4	<LOD	10.2	11.3	1.6						
LC11	14.1	16.9	14.5	15.5	15.6	1.2						
	Avg 11.8				Avg 7.50							
	SD 16				SD 8.0							
	N 5				N 6		N 1				N 1	



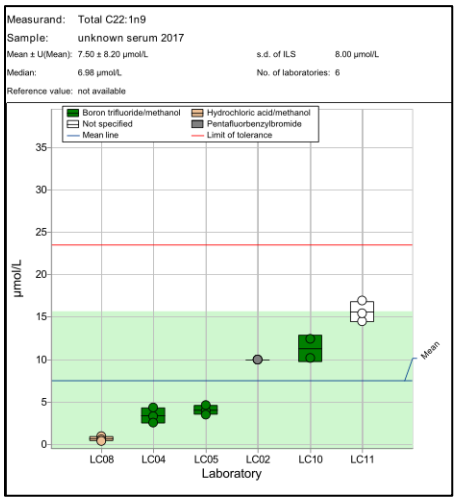
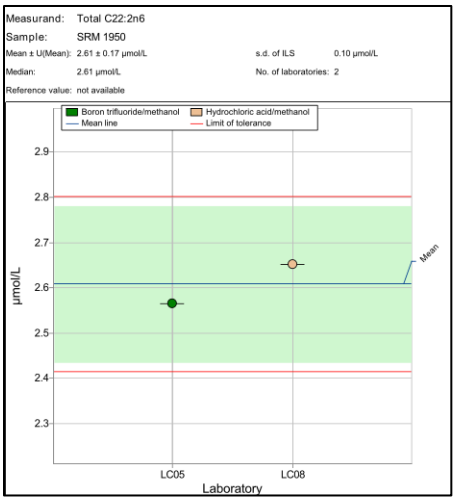


Table A-36: C22:2n6 Docosadienoic acid, µmol/L

	FA		2017 Unknown, FA					FFA	2017 Unknown, FFA				
Lab	SRM 1950		A	B	C	Avg	SD	SRM 1950	A	B	C	Avg	SD
LC05		2.57	1.74	2.27	2.39	2.13	0.34	1.17	1.23	1.23	1.29	1.25	0.032
LC08		2.65	4.02	2.89	3.03	3.31	0.62						
	Avg	2.61				Avg	2.72						
	SD	0.10				SD	1.4						
	N	2				N	2	N 1				N	1



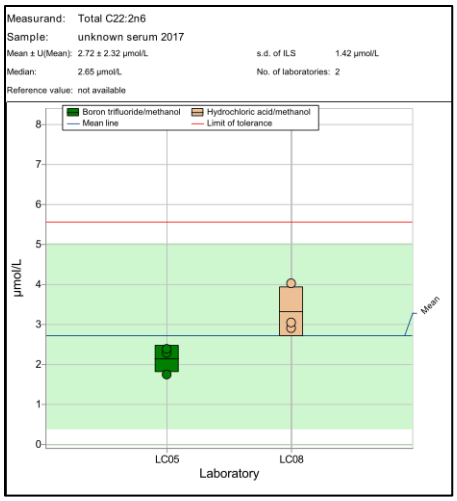
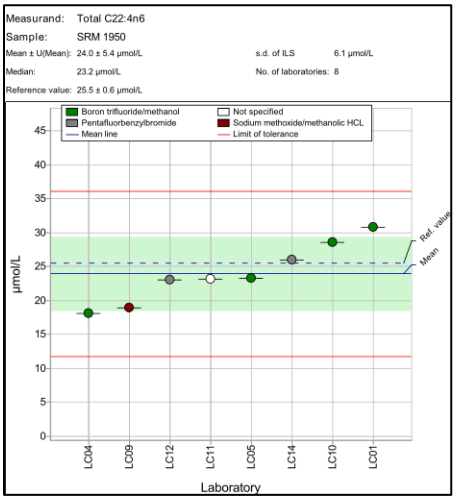
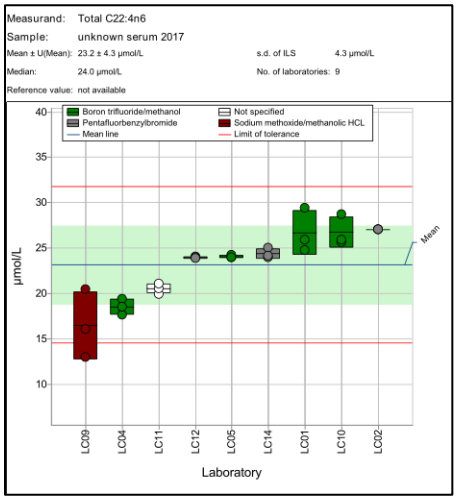
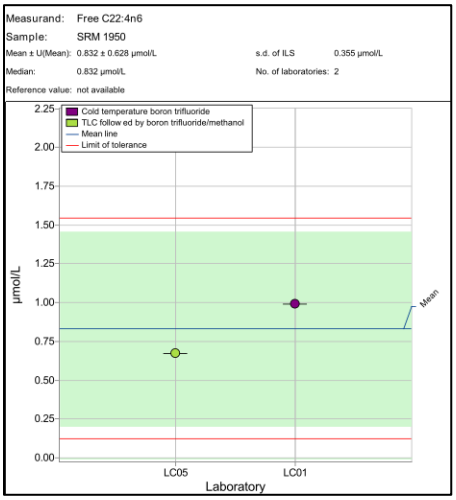


Table A-37: C22:4n6 Docosatetraenoic acid, µmol/L

	FA	2017 Unknown, FA					FFA	2017 Unknown, FFA				
Lab	SRM 1950	A	B	C	Avg	SD	SRM 1950	A	B	C	Avg	SD
NIST	25.5 ± 0.6											
LC01	30.8	24.8	25.9	29.4	26.7	2.4	0.992	0.416	0.587	0.739	0.581	0.16
LC02		27.0	27.0	27.0	27.0	0.0						
LC04	18.1	19.4	17.7	18.5	18.5	0.85						
LC05	23.3	23.9	24.3	24.0	24.0	0.18	0.672	0.740	0.770	0.677	0.729	0.047
LC09	18.9	20.4	13.0	16.0	16.5	3.7		0.000	0.0900	0.0490	0.0463	0.045
LC10	28.5	25.6	25.9	28.7	26.7	1.7						
LC11	23.1	19.9	20.5	21.0	20.5	0.54						
LC12	23.0	24.1	23.9	23.8	23.9	0.13						
LC14	26.0	23.9	25.0	24.1	24.4	0.59						
	Avg 24.0				Avg 23.2		Avg 0.832				Avg 0.452	
	SD 6.1				SD 4.3		SD 0.36				SD 0.61	
	N 8				N 9		N 2				N 3	







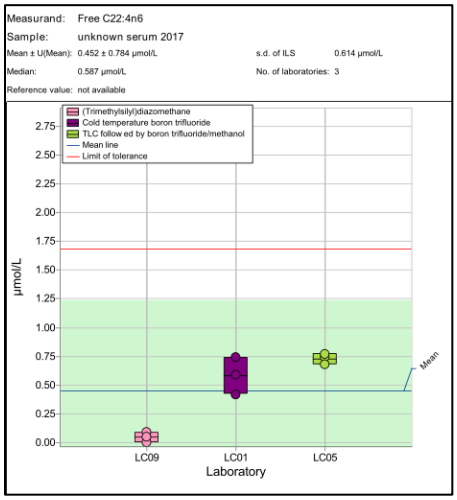
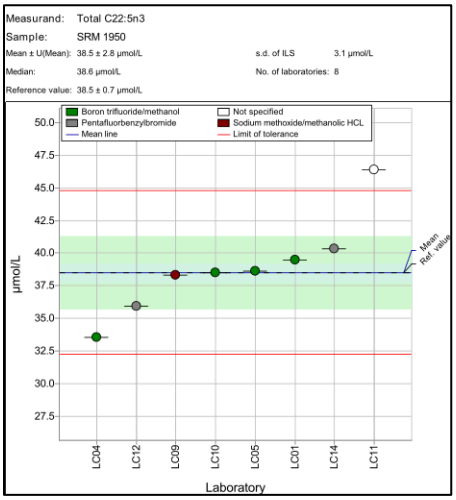
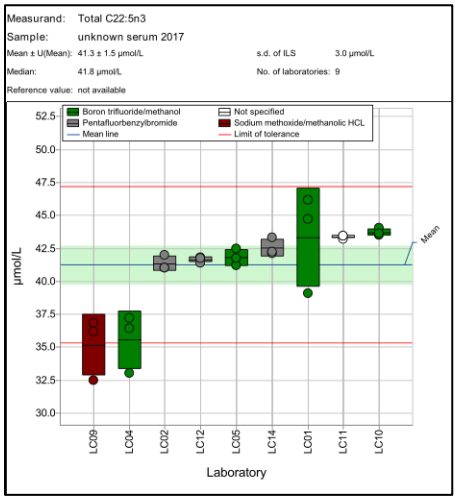
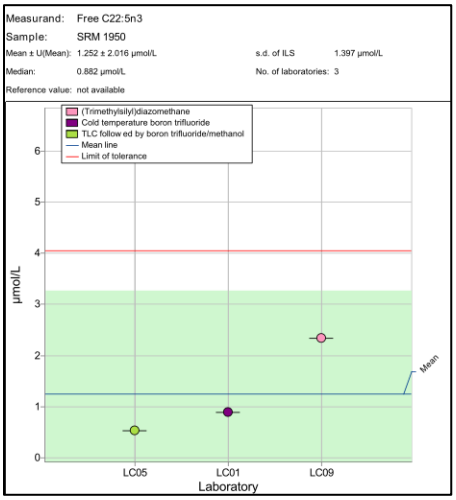


Table A-38: C22:5n3 Docosapentaenoic acid, µmol/L

	FA	2017 Unknown, FA					FFA	2017 Unknown, FFA				
Lab	SRM 1950	A	B	C	Avg	SD	SRM 1950	A	B	C	Avg	SD
NIST	38.5 ± 0.7											
LC01	39.5	39.1	44.7	46.2	43.3	3.7	0.882	1.34	0.489	1.41	1.080	0.51
LC02		41.0	42.0	41.0	41.3	0.58						
LC04	33.5	33.0	37.2	36.4	35.5	2.2						
LC05	38.6	41.2	41.8	42.5	41.8	0.63	0.530	0.652	0.630	0.571	0.618	0.042
LC09	38.3	36.9	36.2	32.5	35.2	2.3	2.34	0.300	0.432	1.26	0.664	0.52
LC10	38.5	43.5	44.0	43.6	43.7	0.28						
LC11	46.4	43.2	43.4	43.4	43.4	0.12						
LC12	36.0	41.8	41.4	41.8	41.6	0.21						
LC14	40.3	42.1	43.3	42.3	42.6	0.67						
	Avg 38.5				Avg 41.3		Avg 1.25				Avg 0.787	
	SD 3.1				SD 3.0		SD 1.4				SD 0.38	
	N 8				N 9		N 3				N 3	







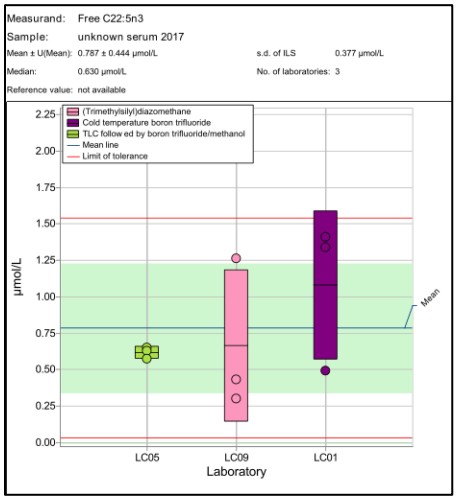
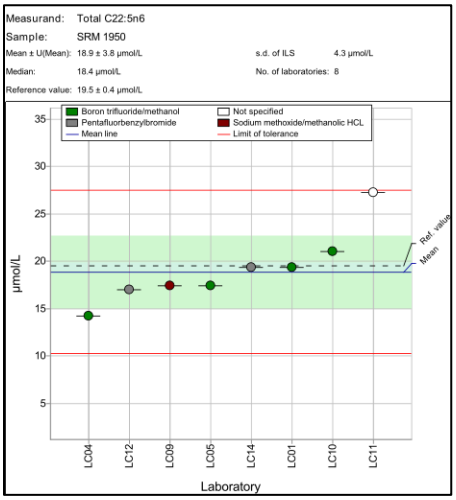
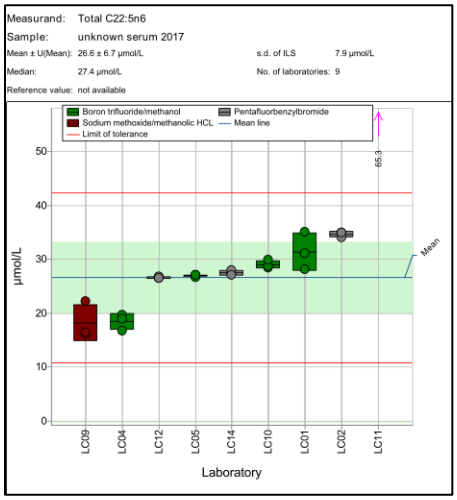
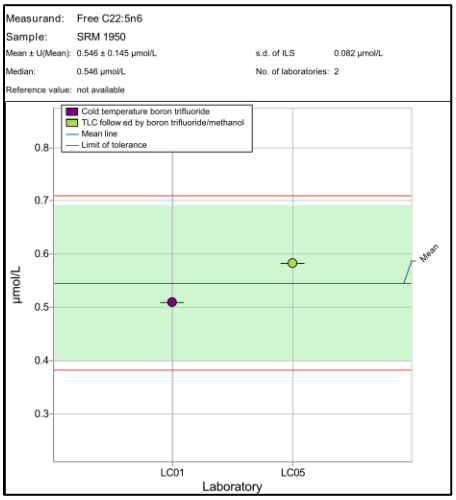


Table A-39: C22:5n6 Docosapentaenoic acid, µmol/L

	FA	2017 Unknown, FA					FFA	2017 Unknown, FFA				
Lab	SRM 1950	A	B	C	Avg	SD	SRM 1950	A	B	C	Avg	SD
NIST	19.5 ± 0.4											
LC01	19.4	35.0	28.1	31.0	31.4	3.5	0.509	0.324	0.282	0.741	0.449	0.25
LC02		35.0	34.0	35.0	34.7	0.58						
LC04	14.2	16.7	19.7	18.9	18.5	1.6						
LC05	17.4	27.0	26.7	27.1	26.9	0.19	0.583	0.620	0.623	1.017	0.753	0.228
LC09	17.4	22.2	16.1	16.4	18.2	3.4		0.755	0.442	0.0700	0.422	0.34
LC10	21.0	28.4	28.8	29.9	29.0	0.80						
LC11	27.3	71.6	65.2	59.2	65.3	6.2						
LC12	17.0	26.4	26.8	26.4	26.6	0.24						
LC14	19.4	27.4	28.0	27.0	27.5	0.48						
	Avg 18.9				Avg 26.6		Avg 0.546				Avg 0.542	
	SD 4.3				SD 7.9		SD 0.082				SD 0.41	
	N 8				N 9		N 2				N 3	







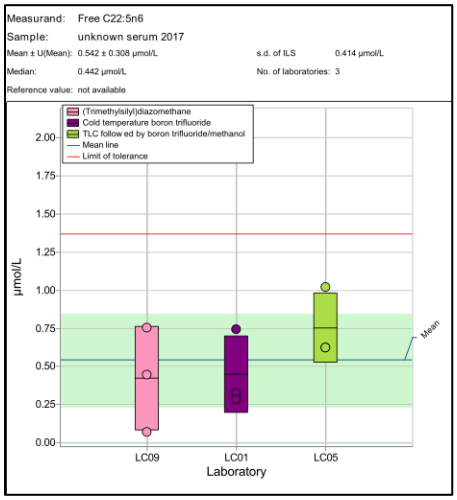
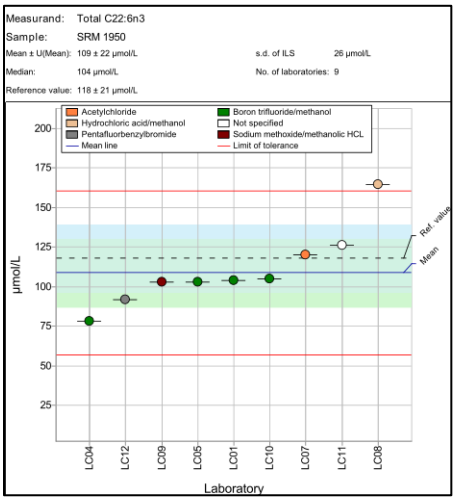
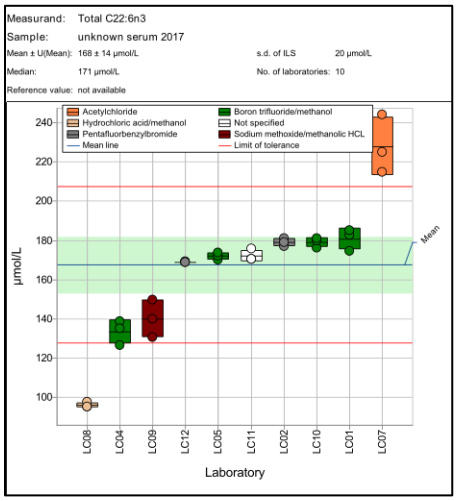
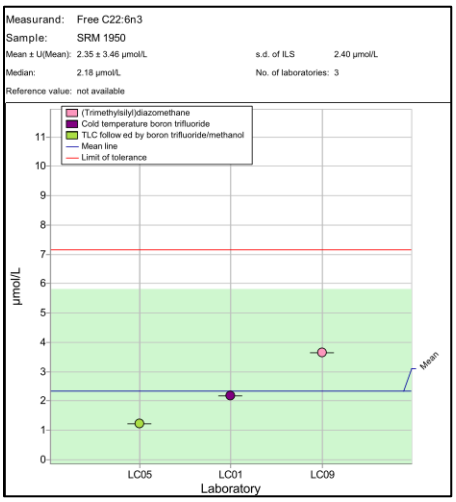


Table A-40: C22:6n3 Docosahexaenoic acid, µmol/L

	FA	2017 Unknown, FA					FFA	2017 Unknown, FFA				
Lab	SRM 1950	A	B	C	Avg	SD	SRM 1950	A	B	C	Avg	SD
NIST	118 ± 21											
LC01	104	175	183	185	181	5.5	2.18	3.16	4.40	4.02	3.86	0.64
LC02		181	177	179	179	2.0						
LC04	78.0	127	139	135	134	6.2						
LC05	103	170	172	174	172	1.8	1.23	2.74	2.82	2.77	2.78	0.042
LC07	120	244	215	225	228	15						
LC08	165	95.2	97.5	95.3	96.0	1.3						
LC09	103	150	140	131	140	9.6	3.64	4.07	4.57	5.24	4.62	0.59
LC10	105	176	180	181	179	2.6						
LC11	126	176	170	170	172	3.0						
LC12	91.7	169	169	169	169	0.29						
	Avg 109				Avg 168		Avg 2.35				Avg 3.75	
	SD 26				SD 20		SD 2.4				SD 1.37	
	N 9				N 10		N 3				N 3	







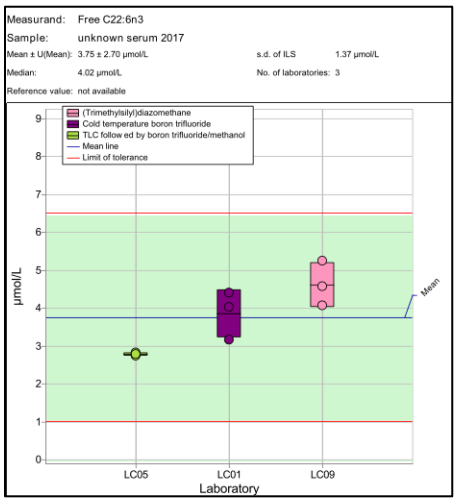
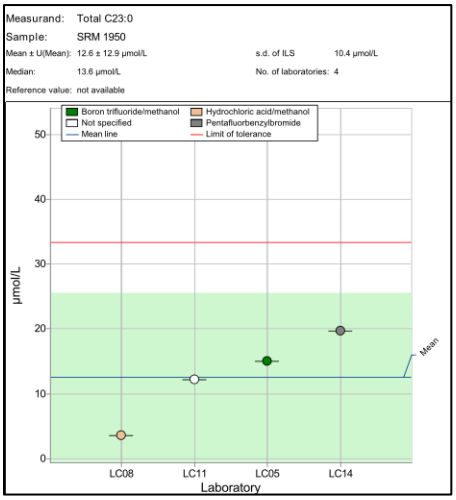


Table A-41: C23:0 Tricosanoic acid, µmol/L

	FA		2017 Unknown, FA					FFA	2017 Unknown, FFA				
Lab	SRM 1950		A	B	C	Avg	SD	SRM 1950	A	B	C	Avg	SD
LC05		15.0	24.2	24.6	24.8	24.5	0.28	0.142	0.229	0.213	0.174	0.205	0.028
LC08		3.52	14.0	13.5	13.2	13.6	0.39						
LC11		12.1	13.5	10.1	12.9	12.2	1.8						
LC14		19.7	31.2	31.0	30.4	30.9	0.41						
	Avg	12.6				Avg	20.3						
	SD	10.4				SD	14.3						
	N	4				N	4	N 1				N	1



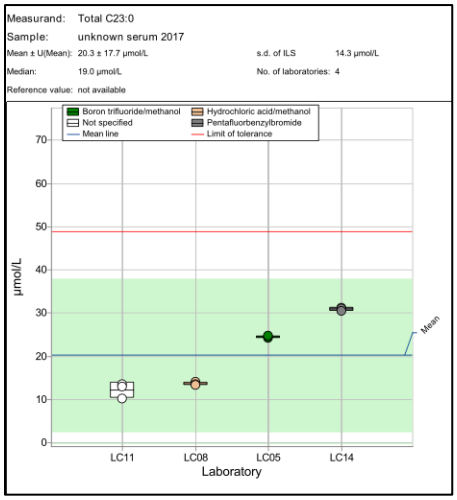
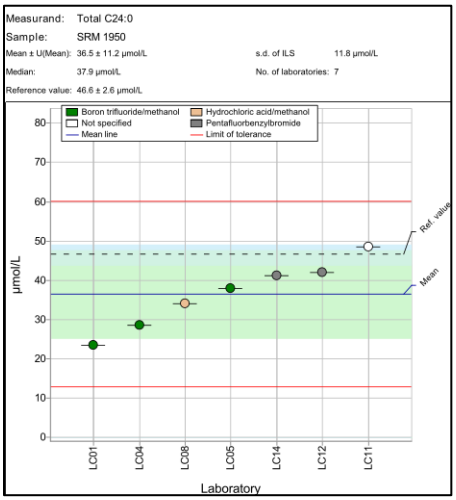
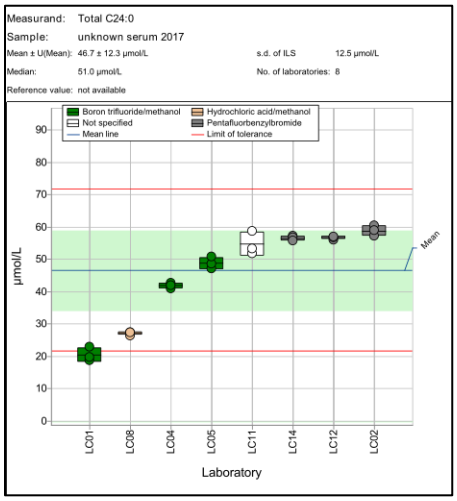
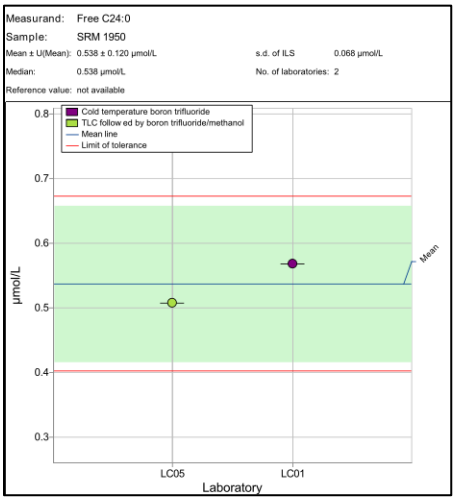


Table A-42: C24:0 Lignoceric acid, µmol/L

	FA		2017 Unknown, FA				FFA		2017 Unknown, FFA			
Lab	SRM 1950		A	B	C	Avg	SD	SRM 1950		A	B	C	Avg	SD
NIST	46.6 ± 2.6													
LC01		23.5	18.7	23.0	19.7	20.4	2.2	0.568		0.661	0.444	0.349	0.485	0.16
LC02			60.4	57.3	58.9	58.9	1.6							
LC04		28.6	40.9	42.7	42.0	41.9	0.90							
LC05		37.9	47.2	48.6	50.8	48.8	1.8	0.507		0.467	0.529	0.601	0.532	0.067
LC08		34.0	27.5	26.5	27.3	27.1	0.52							
LC11		48.4	58.9	51.9	53.4	54.7	3.7							
LC12		41.9	56.1	56.8	57.0	56.7	0.48							
LC14		41.1	57.3	56.7	55.7	56.6	0.83							
	Avg	36.5				Avg	46.7	Avg	0.538				Avg	0.509
	SD	12				SD	13	SD	0.068				SD	0.18
	N	7				N	8	N	2				N	2







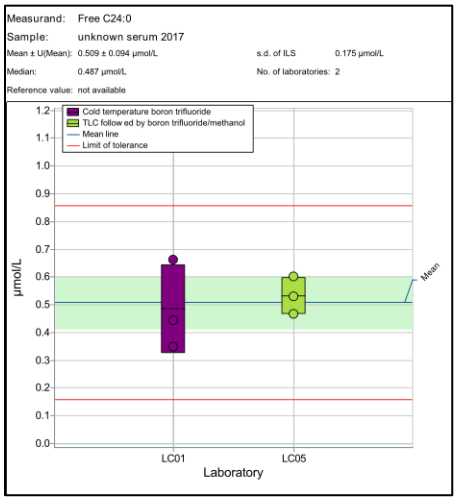
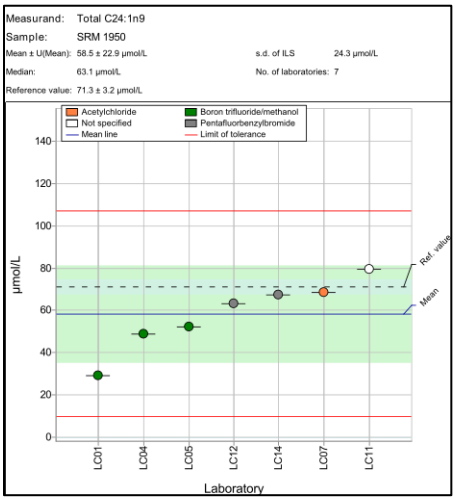
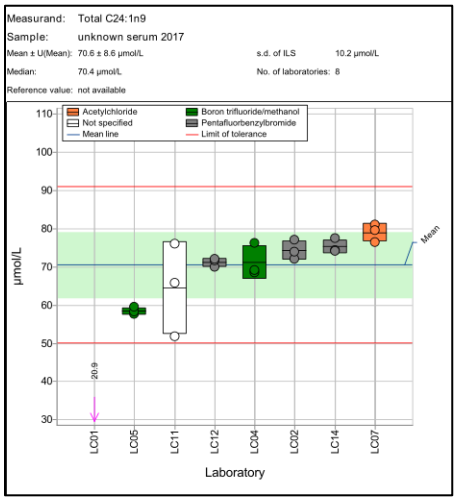
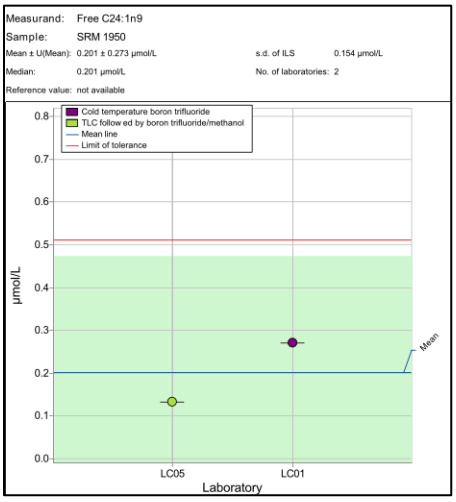


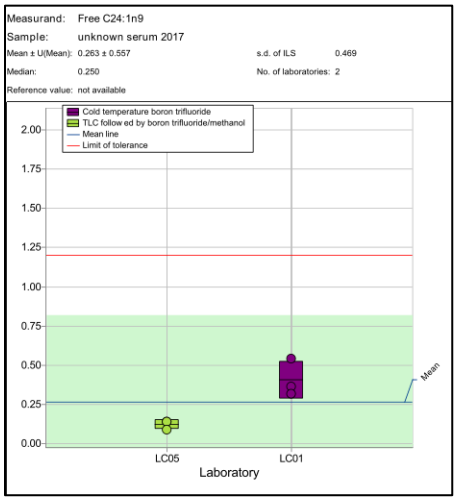
Table A-43: C24:1n9 Nervonic acid, µmol/L

	FA		2017 Unknown, FA				FFA		2017 Unknown, FFA			
Lab	SRM 1950		A	B	C	Avg	SD	SRM 1950	A	B	C	Avg	SD
NIST	71.3 ±	3.2											
LC01		29.2	22.9	20.2	19.7	20.9	1.7	0.271	0.540	0.362	0.314	0.405	0.12
LC02			77.0	72.0	74.0	74.3	2.5						
LC04		49.0	68.5	69.1	76.3	71.3	4.4						
LC05		52.2	57.6	58.4	59.5	58.5	1.0	0.132	0.139	0.138	0.088	0.122	0.029
LC07		68.7	81.0	76.4	79.6	79.0	2.4						
LC11		79.7	51.9	65.8	76.1	64.6	12						
LC12		63.1	69.9	71.7	72.0	71.2	1.1						
LC14		67.4	77.5	74.3	74.2	75.3	1.8						
	Avg	58.5				Avg	70.6	Avg	0.201			Avg	0.263
	SD	24				SD	10	SD	0.15			SD	0.47
	N	7				N	8	N	2			N	2









Appendix B: Free Fatty Acids and Fatty Acid Methyl Esters in Solutions

List of Tables

Acronyms Used in Tables

Avg	Mean
FA	Fatty acid
FAME	Fatty acid methyl ester
FFA	Free fatty acid
Lab	Participant code
N	Number of quantitative results
SD	Standard deviation

Table B-1: C16:1n5 *cis*-11-hexadecenoic acid, µmol/L

	FFA Solutions					FA/FAME Solution D	
Lab	A	B	C	Avg	SD	As FA	As FAME
LC05						51.1	

Table B-2: C16:1n5t *trans*-11-Hexadecenoic acid, µmol/L

	FFA Solutions					FA/FAME Solution D	
Lab	A	B	C	Avg	SD	As FA	As FAME
LC05						4.07	

Table B-3: C16:1n10 Sapienic acid, µmol/L

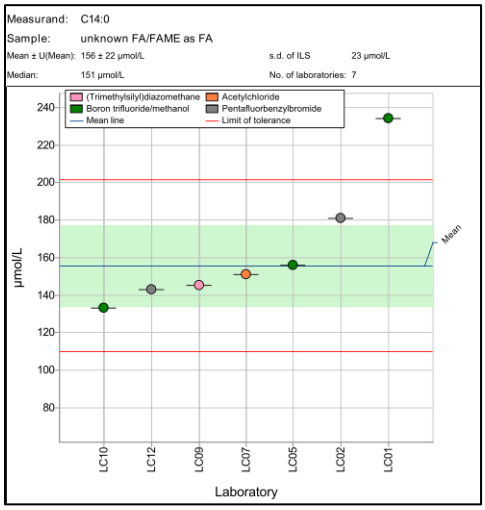
	FFA Solutions					FA/FAME Solution D	
Lab	A	B	C	Avg	SD	As FA	As FAME
LC05						24.0	

Table B-4: C18:1n7t *trans*-Vaccenic acid, µmol/L

	FFA Solutions					FA/FAME Solution D	
Lab	A	B	C	Avg	SD	As FA	As FAME
LC12						30.1	

Table B-5: C14:0 Myristic acid, µmol/L

	FFA Solutions					FA/FAME Solution D	
Lab	A	B	C	Avg	SD	As FA	As FAME
LC01						234	
LC02						181	
LC05						156	163
LC07						151	168
LC09						145	
LC10						133	126
LC11							148
LC12						143	
						Avg 156	Avg 151
						SD 23	SD 34
						N 7	N 4



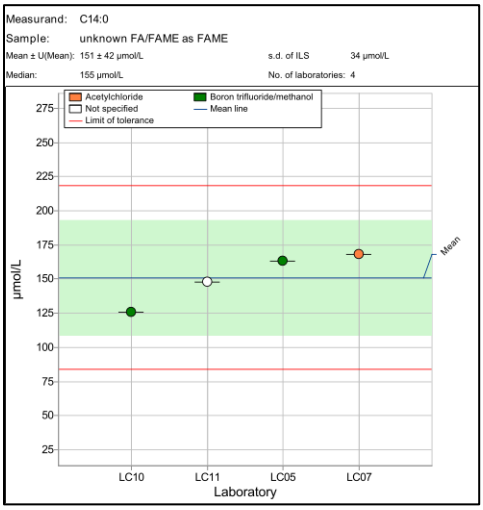
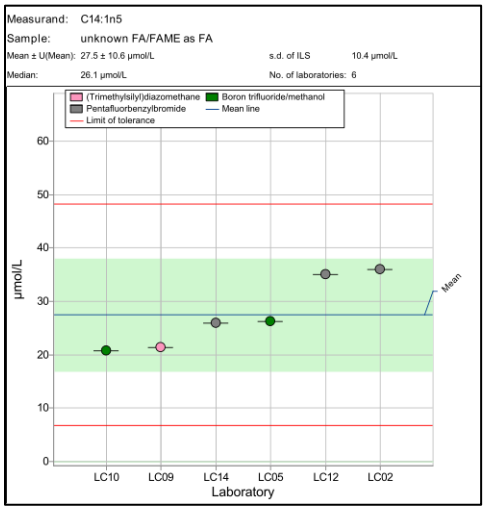


Table B-6: C14:1n5 Myristoleic acid, µmol/L

	FFA Solutions					FA/FAME Solution D			
Lab	A	B	C	Avg	SD	As FA		As FAME	
LC02							36		
LC05							26		28
LC09							21		
LC10							21		20
LC11									14
LC12							35		
LC14							26		
						Avg	27.5	Avg	20.5
						SD	10	SD	14
						N	6	N	3



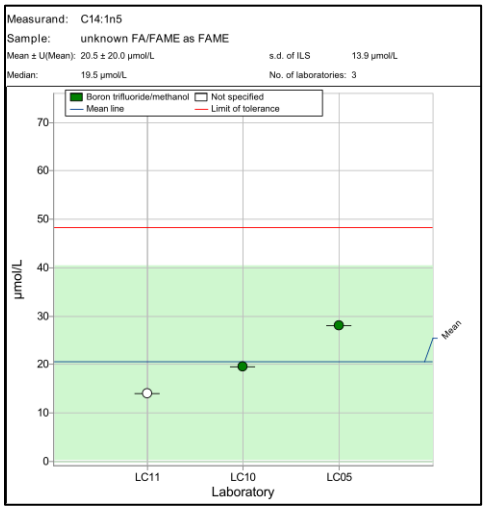
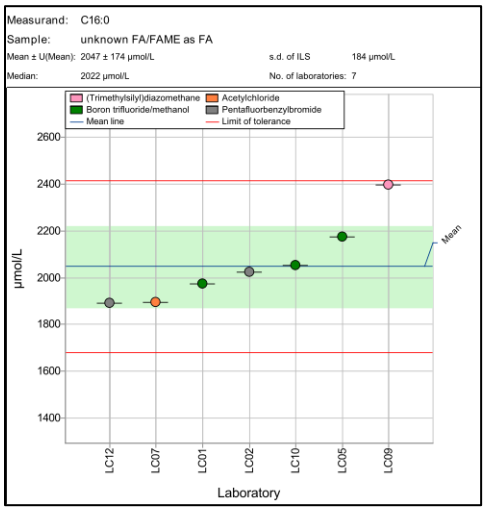


Table B-7: C16:0 Palmitic acid, µmol/L

	FFA Solutions					FA/FAME Solution D	
Lab	A	B	C	Avg	SD	As FA	As FAME
LC01						1974	
LC02						2022	
LC05						2173	2186
LC07						1895	2091
LC09						2397	
LC10						2051	1944
LC11							2283
LC12						1890	
						Avg 2047	Avg 2126
						SD 184	SD 216
						N 7	N 4



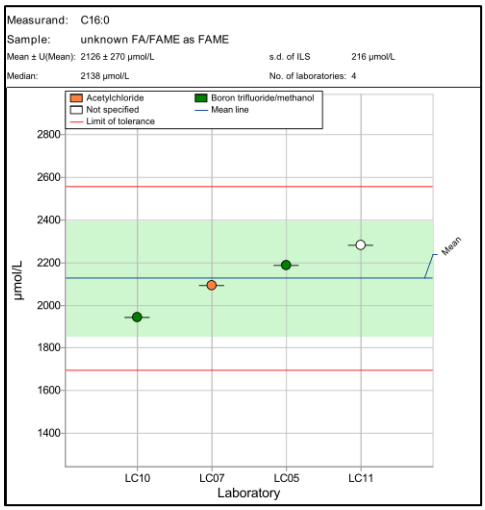
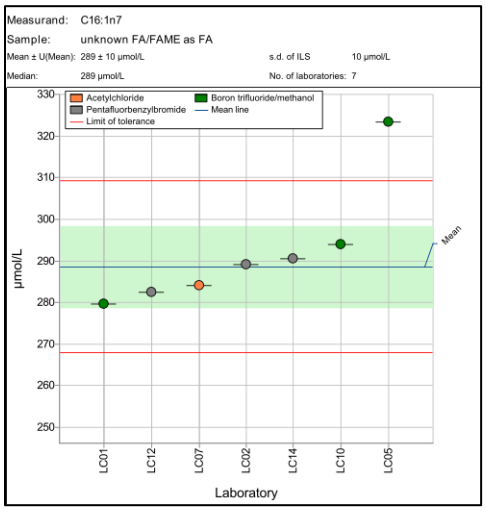


Table B-8: C16:1n7 Palmitoleic acid, µmol/L

	FFA Solutions					FA/FAME Solution D			
Lab	A	B	C	Avg	SD	As FA		As FAME	
LC01						280			
LC02						289			
LC05						323		326	
LC07						284		315	
LC10						294		279	
LC11								337	
LC12						282			
LC14						290			
						Avg	289	Avg	314
						SD	10	SD	25
						N	7	N	4



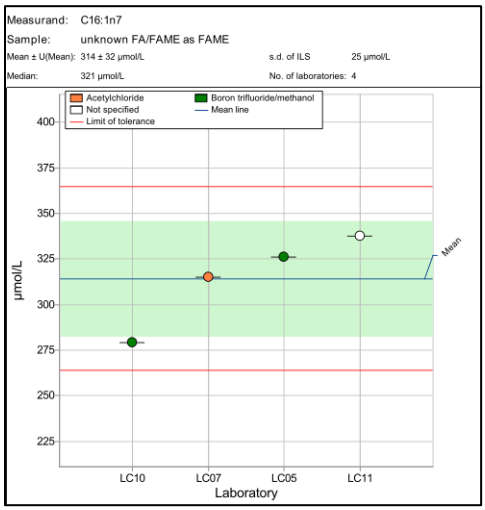


Table B-9: C16:1n7t Palmitelaidic acid, µmol/L

	FFA Solutions					FA/FAME Solution D	
Lab	A	B	C	Avg	SD	As FA	As FAME
LC01						12.4	
LC09						22.0	
LC12						5.48	
						Avg 13.3	
						SD 17	
						N 3	

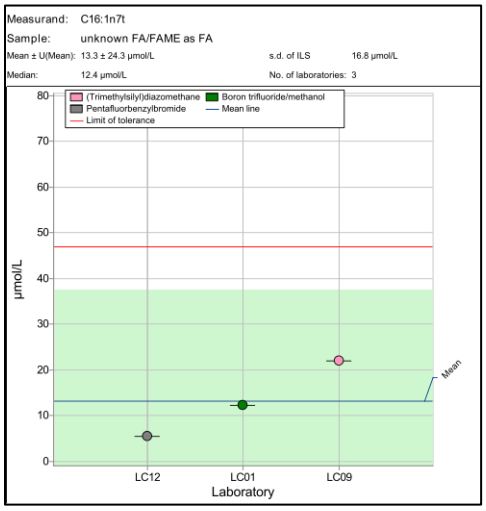
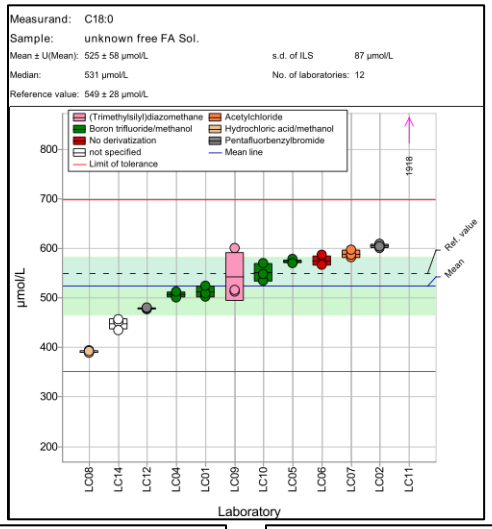
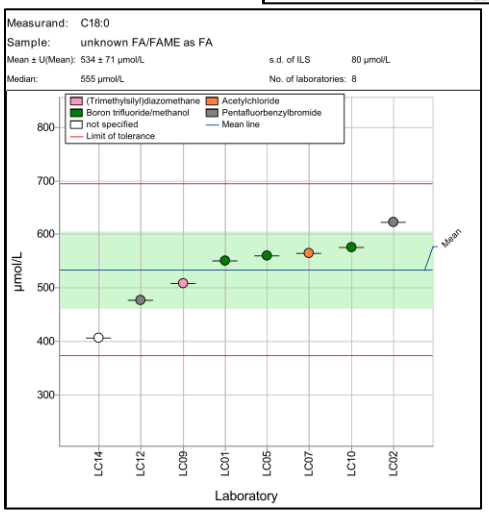


Table B-10: C18:0 Stearic acid, µmol/L

	FFA Solutions					FA/FAME Solution D			
Lab	A	B	C	Avg	SD	As FA		As FAME	
LC01	501	508	524	511	12		550		
LC02	600	609	604	604	4.5		623		
LC04	506	500	512	506	6.1				
LC05	572	577	570	573	4.0		561		556
LC06	572	586	567	575	10				
LC07	581	586	597	588	8.2		564		536
LC08	393	388	391	391	2.7				
LC09	599	513	515	542	49		509		
LC10	534	570	547	550	18.2		576		549
LC11	1709	2338	1705	1918	364				652
LC12	477	478	481	478	2.08		478		
LC14	452	456	435	447	11.2		406		
				Avg	525	Avg	534	Avg	559
				SD	87	SD	80	SD	29
				N	12	N	8	N	4





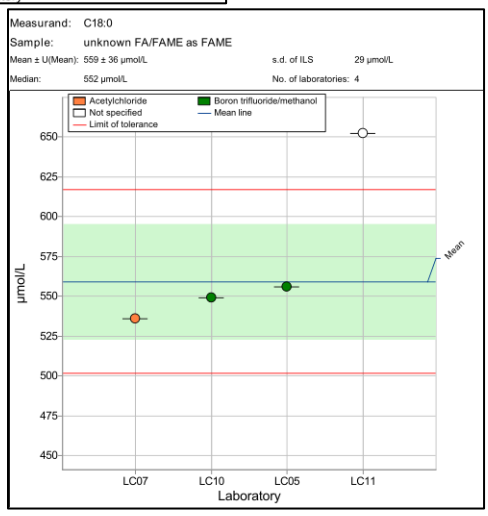
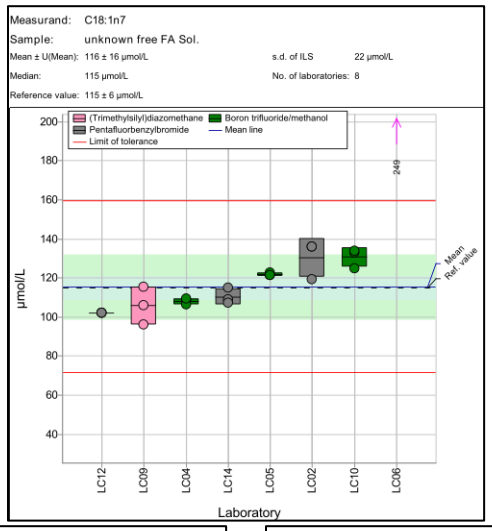
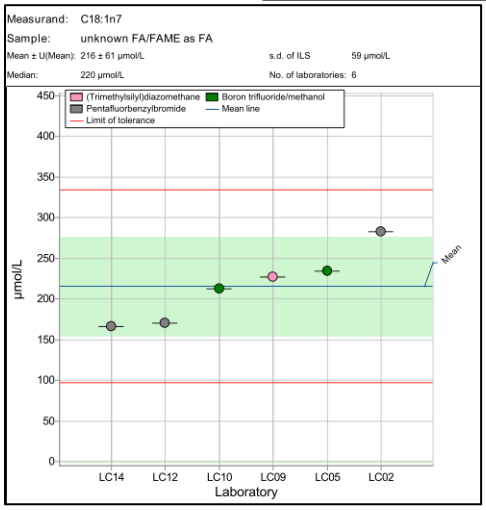


Table B-11: C18:1n7 *cis*-Vaccenic acid, µmol/L

	FFA Solutions					FA/FAME Solution D			
Lab	A	B	C	Avg	SD	As FA		As FAME	
LC02	136	136	119	130	9.8		283		
LC04	108	106	109	108	1.6				
LC05	121	123	121	122	0.89		234		236
LC06	258	255	234	249	13				
LC09	115	106	96	106	10		227		
LC10	125	133	134	131	4.9		213		203
LC12	102	102	102	102	0.10		170		
LC14	109	107	115	110	3.9		166		
				Avg	116	Avg	216	Avg	220
				SD	22	SD	59	SD	37
				N	8	N	6	N	2





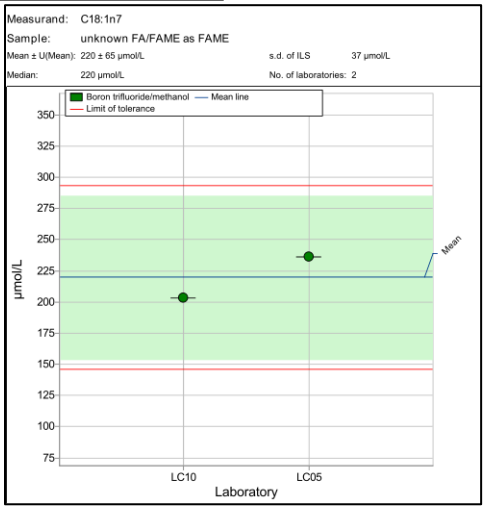
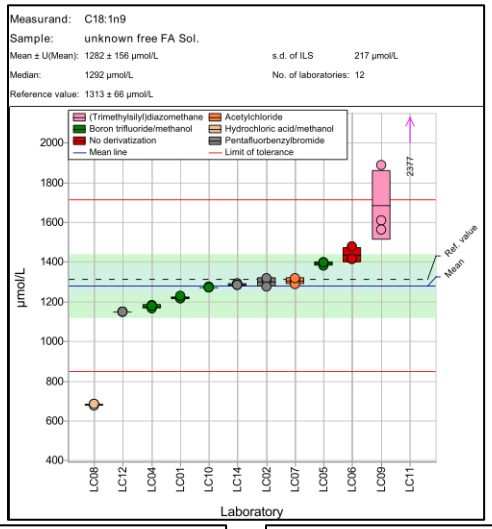
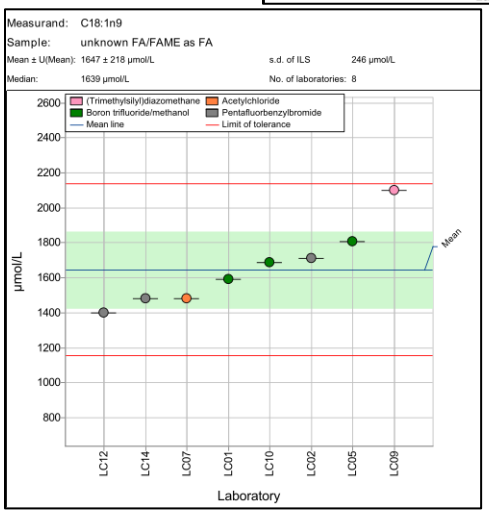


Table B-12: C18:1n9 Oleic acid, µmol/L

	FFA Solutions					FA/FAME Solution D			
Lab	A	B	C	Avg	SD	As FA		As FAME	
LC01	1215	1218	1227	1220	6.5		1592		
LC02	1303	1319	1274	1299	23		1711		
LC04	1183	1165	1178	1175	9.4				
LC05	1379	1398	1398	1391	10.9		1805		1816
LC06	1477	1414	1415	1436	36				
LC07	1306	1289	1319	1305	15		1482		1654
LC08	683	676	685	682	4.8				
LC09	1885	1606	1562	1685	175		2102		
LC10	1277	1273	1269	1273	4.0		1685		1605
LC11	2393	2304	2436	2377	67				2179
LC12	1150	1150	1150	1150	0.0		1400		
LC14	1292	1282	1282	1285	6.0		1481		
				Avg	1282	Avg	1647	Avg	1814
				SD	217	SD	246	SD	360
				N	12	N	8	N	4





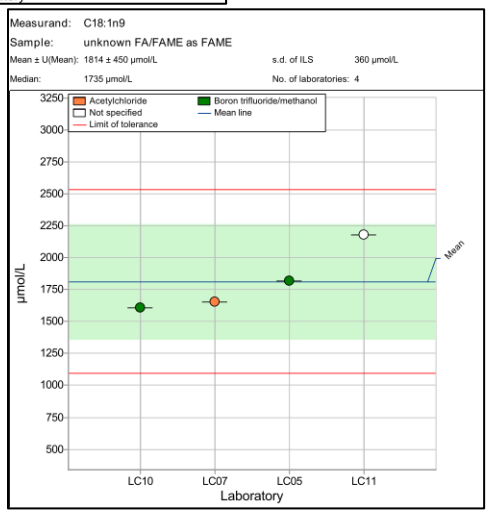


Table B-13: C18:1n9t Elaidic acid, µmol/L

	FFA Solutions					FA/FAME Solution D			
Lab	A	B	C	Avg	SD	As FA		As FAME	
LC01						105			
LC11								308	
LC12						31.5			
						Avg	68.2		
						SD	82		
						N	2	N	1

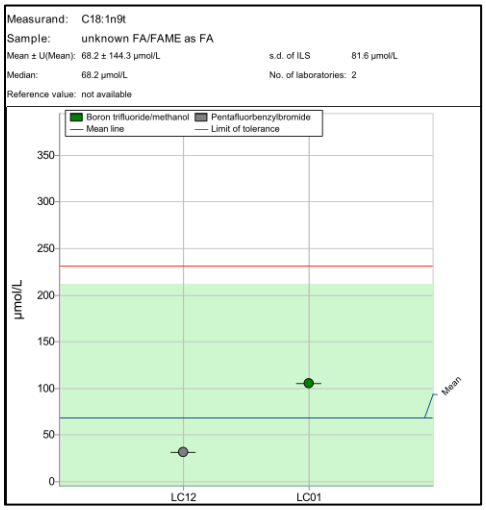
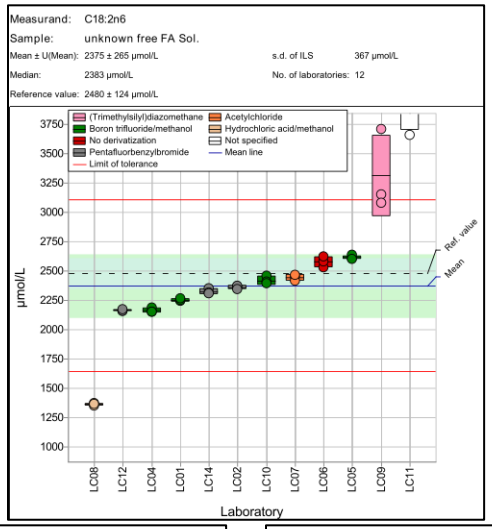
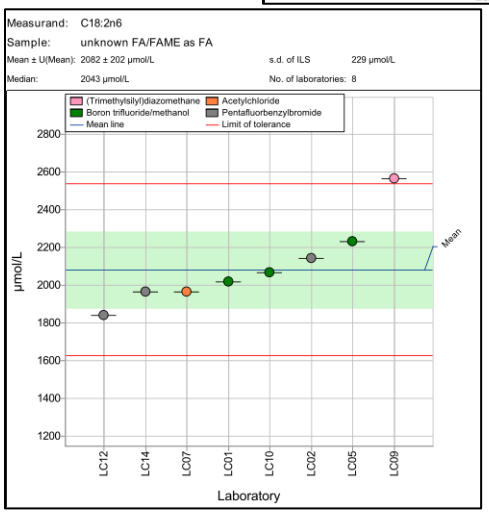


Table B-14: C18:2n6 Linoleic acid, µmol/L

	FFA Solutions					FA/FAME Solution D			
Lab	A	B	C	Avg	SD	As FA		As FAME	
LC01	2241	2250	2266	2252	13		2017		
LC02	2362	2374	2342	2359	16		2144		
LC04	2186	2153	2152	2164	20				
LC05	2607	2635	2602	2615	18		2233		2250
LC06	2530	2581	2620	2577	45				
LC07	2447	2416	2466	2443	25		1966		2176
LC08	1370	1351	1366	1363	9.9				
LC09	3706	3153	3076	3312	343		2567		
LC10	2459	2404	2390	2418	36		2068		1970
LC11	4122	3661	4114	3965	264				2558
LC12	2160	2160	2170	2163	5.8		1840		
LC14	2351	2313	2308	2324	23.7		1964		
				Avg	2375	Avg	2082	Avg	2239
				SD	367	SD	229	SD	457
				N	12	N	8	N	4





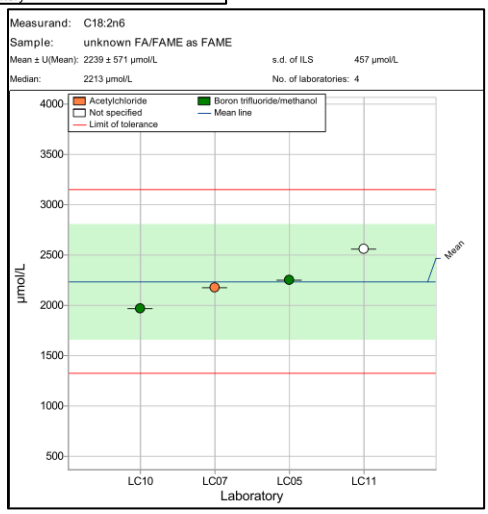


Table B-15: C18:2n6t,9t Linoelaidic acid, µmol/L

	FFA Solutions					FA/FAME Solution D	
Lab	A	B	C	Avg	SD	As FA	As FAME
LC01						48.4	
LC12						2.26	
						Avg 25.3	
						SD 51	
						N 2	

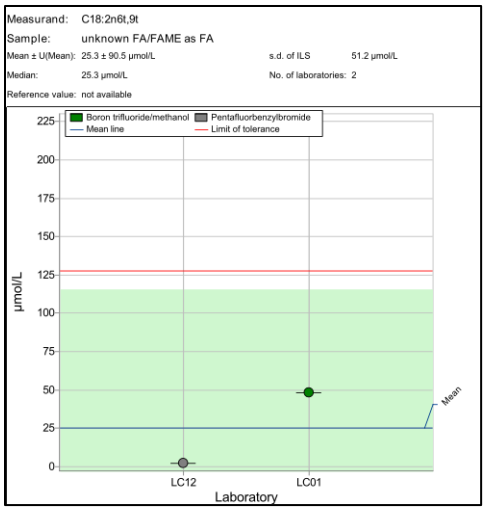
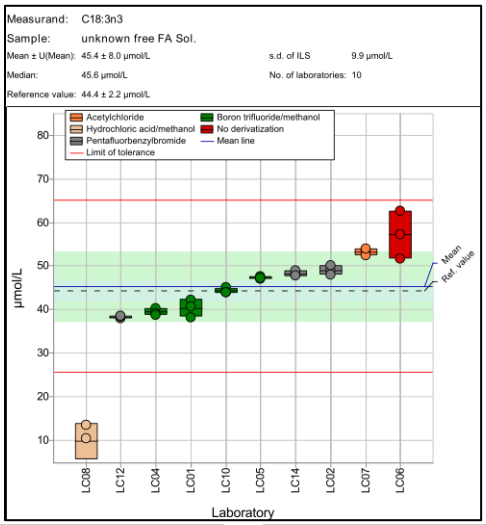
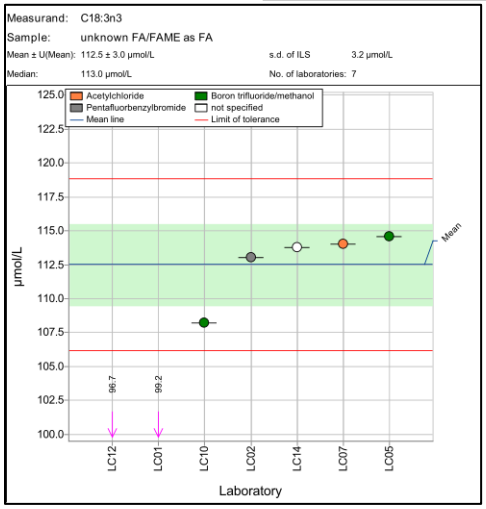


Table B-16: C18:3n3 α-Linolenic acid, µmol/L

	FFA Solutions					FA/FAME Solution D			
Lab	A	B	C	Avg	SD	As FA		As FAME	
LC01	42.2	38.1	40.7	40.3	2.0		99.2		
LC02	49.0	50.0	48.0	49.0	1.0		113		
LC04	40.3	39.2	38.8	39.4	0.77				
LC05	47.1	47.7	47.1	47.3	0.32		115		116
LC06	51.6	57.2	62.6	57.2	5.5				
LC07	53.5	52.4	53.9	53.3	0.78		114		124
LC08	13.5	5.23	10.4	9.70	4.2				
LC10	45.0	44.0	44.0	44.3	0.58		108		103
LC11									42.5
LC12	37.9	38.3	38.4	38.2	0.27		96.7		
LC14	48.0	49.0	47.7	48.2	0.69		114		
				Avg	45.4	Avg	113	Avg	100
				SD	9.9	SD	3.2	SD	28
				N	10	N	7	N	4





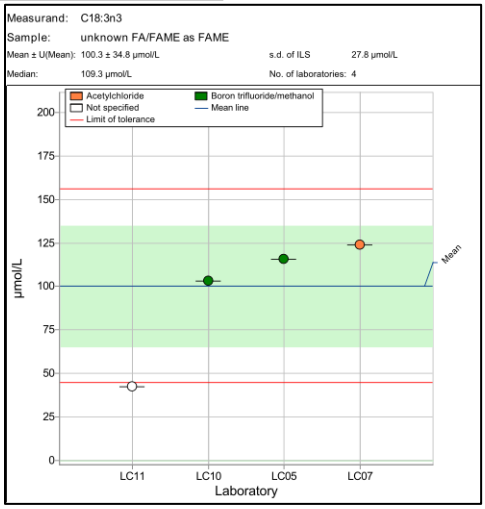
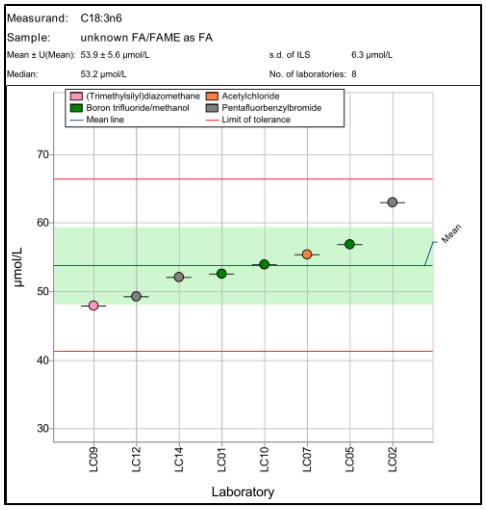


Table B-17: C18:3n6 γ-Linolenic acid, µmol/L

	FFA Solutions					FA/FAME Solution D			
Lab	A	B	C	Avg	SD	As FA		As FAME	
LC01						52.5			
LC02						63.0			
LC05						56.8		57.6	
LC07						55.4		61.9	
LC09						47.9			
LC10						53.9		51.3	
LC11								0.0	
LC12						49.2			
LC14						52.0			
						Avg	53.9	Avg	53.8
						SD	6.3	SD	14
						N	8	N	4



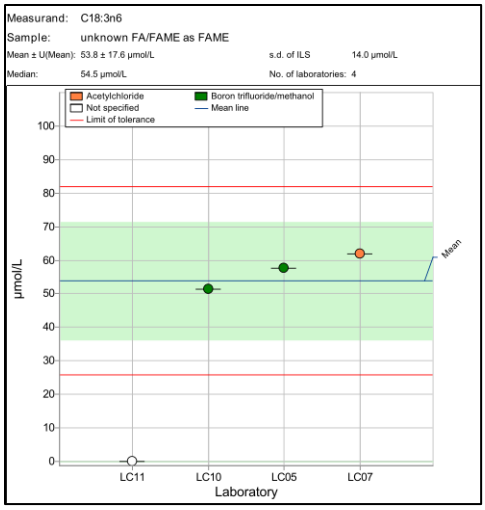
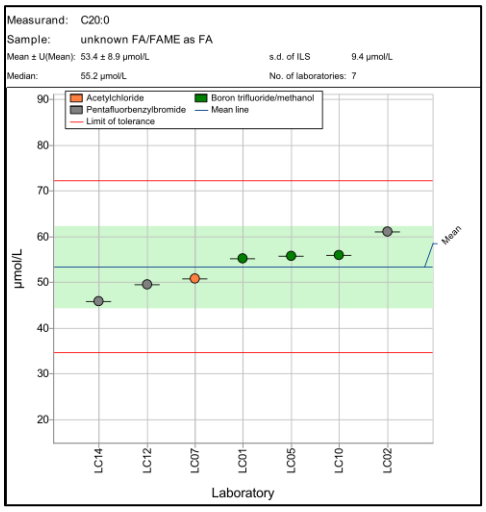


Table B-18: C20:0 Arachidic acid, µmol/L

	FFA Solutions					FA/FAME Solution D	
Lab	A	B	C	Avg	SD	As FA	As FAME
LC01						55.2	
LC02						61.0	
LC05						55.8	56.0
LC07						50.8	54.8
LC10						56.0	53.1
LC11							47.5
LC12						49.5	
LC14						45.7	
						Avg 53.4	Avg 52.8
						SD 9.4	SD 3.8
						N 7	N 4



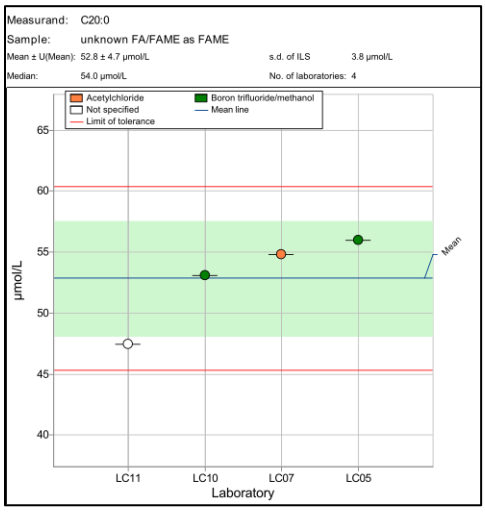
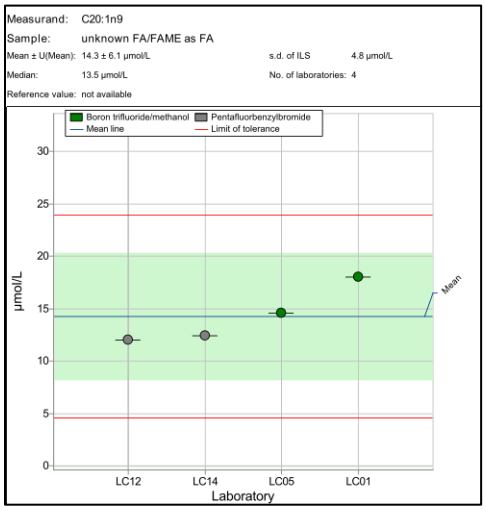


Table B-19: C20:1n9 11-Eicosenoic acid, µmol/L

	FFA Solutions					FA/FAME Solution D			
Lab	A	B	C	Avg	SD	As FA		As FAME	
LC01						18.0			
LC05						14.6		14.0	
LC11								7.38	
LC12						12.0			
LC14						12.4			
						Avg	14.3	Avg	10.7
						SD	4.8	SD	7.3
						N	4	N	2



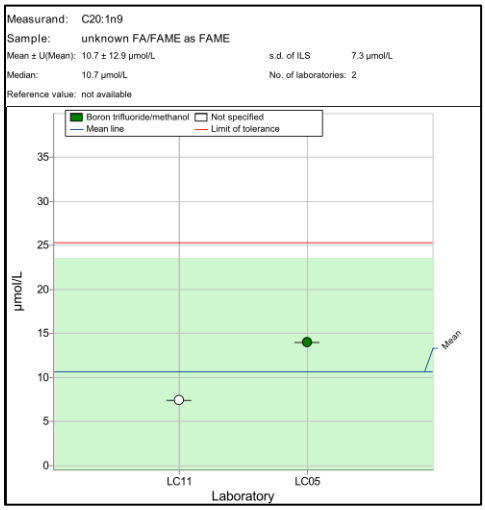
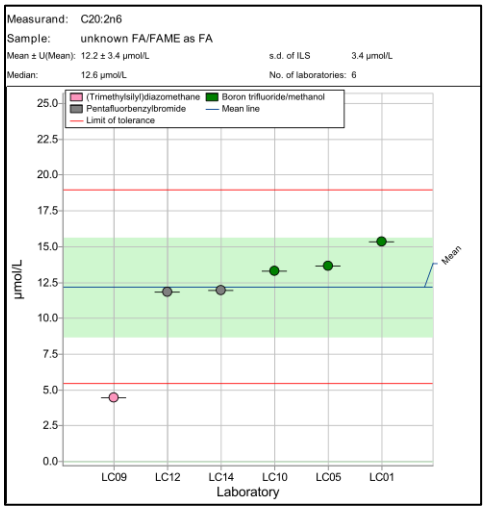


Table B-20: C20:2n6 11,14-Eicosadienoic acid, µmol/L

	FFA Solutions					FA/FAME Solution D	
Lab	A	B	C	Avg	SD	As FA	As FAME
LC01						15.3	
LC05						13.6	13.7
LC09						4.43	
LC10						13.3	12.7
LC11							6.70
LC12						11.8	
LC14						12.0	
						Avg 12.2	Avg 11.0
						SD 3.4	SD 5.0
						N 6	N 3



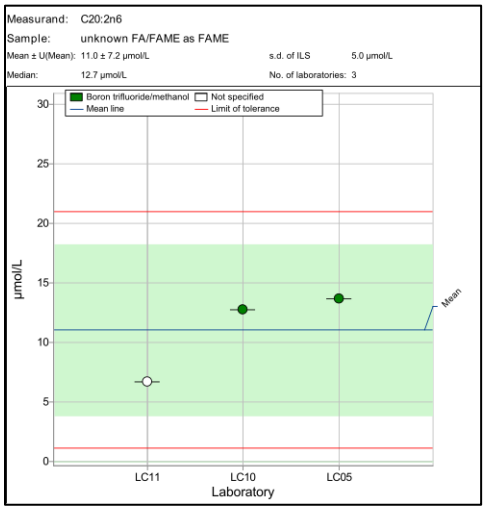
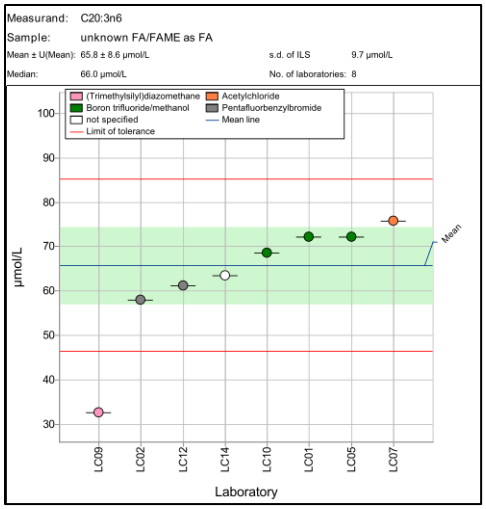


Table B-21: C20:3n6 homo-γ-Linolenic acid, µmol/L

	FFA Solutions					FA/FAME Solution D			
Lab	A	B	C	Avg	SD	As FA		As FAME	
LC01						72.1			
LC02						58.0			
LC05						72.2		72.5	
LC07						75.8		83.5	
LC09						32.7			
LC10						68.5		65.5	
LC11								72.7	
LC12						61.3			
LC14						63.5			
						Avg	65.8	Avg	73.6
						SD	9.7	SD	16
						N	8	N	4



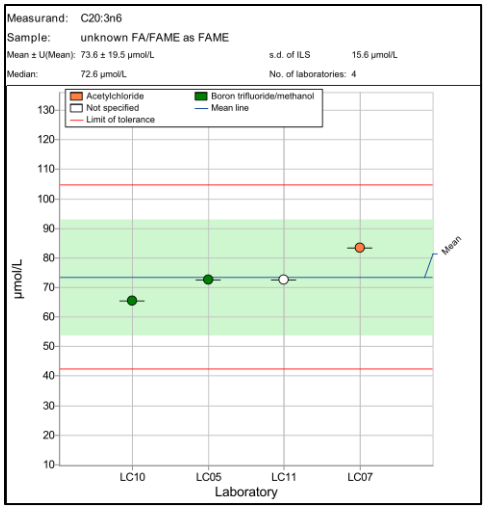
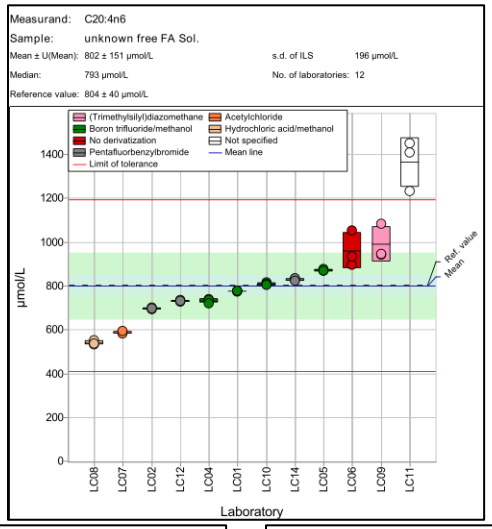
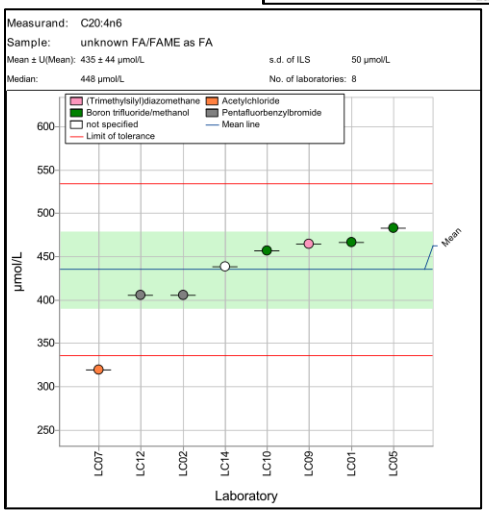


Table B-22: C20:4n6 Arachidonic acid, µmol/L

	FFA Solutions					FA/FAME Solution D			
Lab	A	B	C	Avg	SD	As FA		As FAME	
LC01	775	778	777	777	1.4		467		
LC02	701	693	698	697	4.0		406		
LC04	739	736	721	732	9.6				
LC05	870	876	867	871	4.4		483		487
LC06	1053	896	933	961	82				
LC07	588	583	592	588	4.5		319		346
LC08	551	534	538	541	8.7				
LC09	1084	943	947	991	81		464		
LC10	815	808	802	808	6.5		457		437
LC11	1449	1233	1408	1364	115				608
LC12	733	728	732	731	3.0		405		
LC14	827	835	822	828	6.6		438		
				Avg	802	Avg	435	Avg	469
				SD	196	SD	50	SD	202
				N	12	N	8	N	4





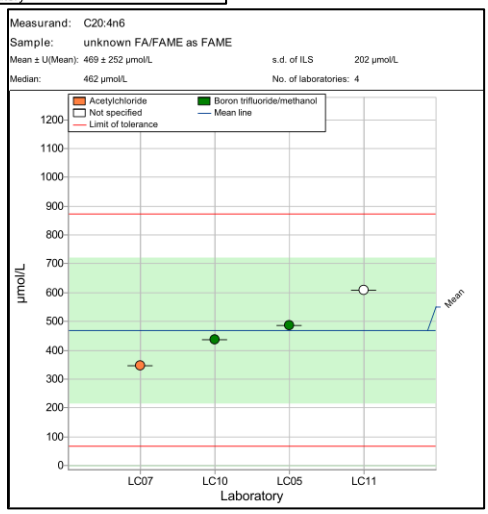
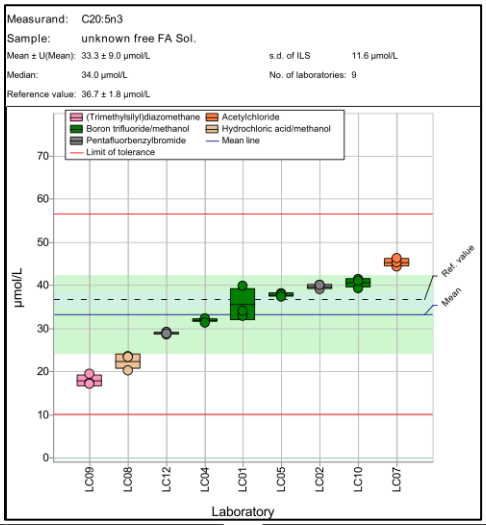
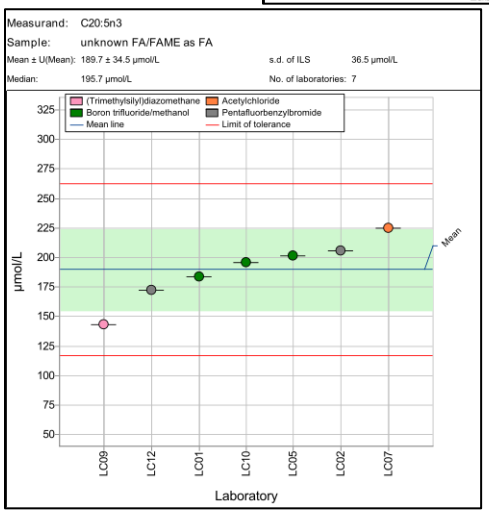


Table B-23: C20:5n3 Eicosapentaenoic acid, µmol/L

	FFA Solutions					FA/FAME Solution D			
Lab	A	B	C	Avg	SD	As FA		As FAME	
LC01	32.9	34.1	39.7	35.6	3.7		184		
LC02	40.0	39.0	40.0	39.7	0.58		206		
LC04	32.0	32.2	31.3	31.8	0.44				
LC05	38.1	38.0	37.2	37.8	0.47		202		204
LC07	44.3	45.3	46.3	45.3	1.0		225		254
LC08	23.5	23.3	20.3	22.4	1.8				
LC09	19.4	17.2	17.1	17.9	1.3		143		
LC10	39.3	41.3	41.0	40.5	1.08		196		187
LC11									247
LC12	28.5	29.1	28.7	28.8	0.32		172		
				Avg	33.3	Avg	190	Avg	223
				SD	12	SD	36	SD	38
				N	9	N	7	N	4





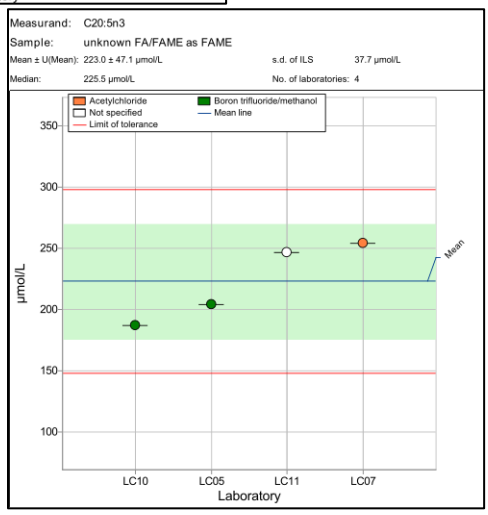
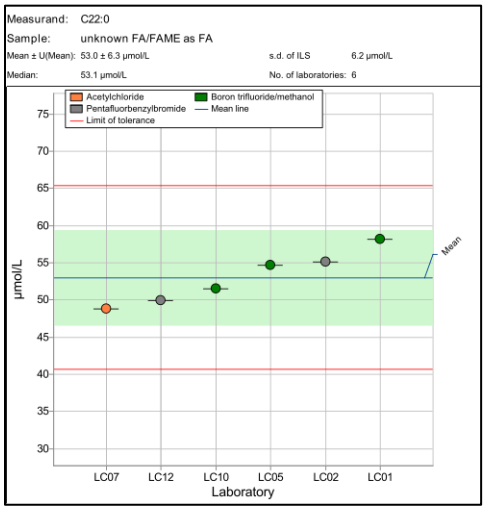


Table B-24: C22:0 Docosanoic acid, µmol/L

	FFA Solutions					FA/FAME Solution D			
Lab	A	B	C	Avg	SD	As FA		As FAME	
LC01						58.2			
LC02						55.1			
LC05						54.7		55.5	
LC07						48.8		55.0	
LC10						51.5		49.4	
LC11								36.3	
LC12						49.9			
						Avg	53.0	Avg	49.0
						SD	6.2	SD	12
						N	6	N	4



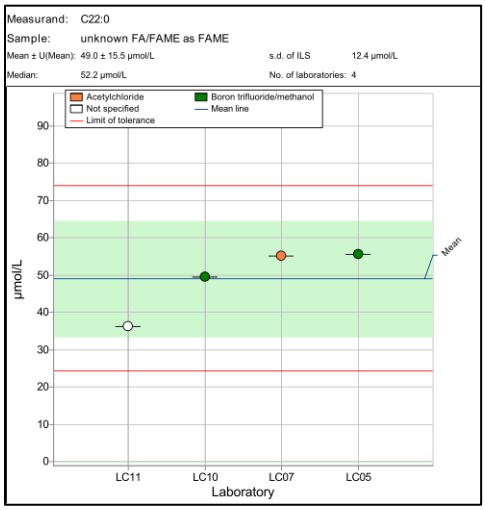
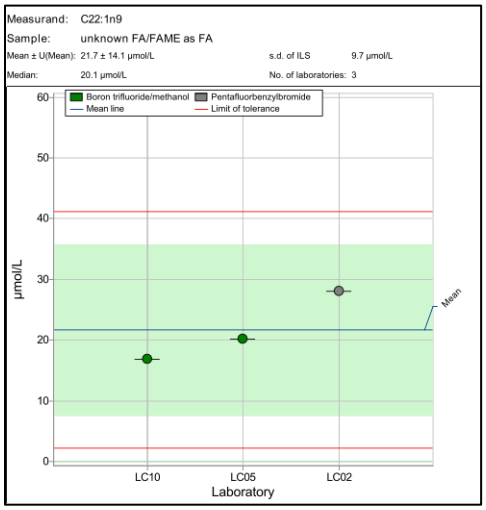


Table B-25: C22:1n9 Docosenoic acid, µmol/L

	FFA Solutions					FA/FAME Solution D			
Lab	A	B	C	Avg	SD	As FA		As FAME	
LC01									
LC02							28.0		
LC05							20.1		14.5
LC10							16.9		16.2
LC11									5.26
						Avg	21.7	Avg	12.0
						SD	9.7	SD	7.9
						N	3	N	3



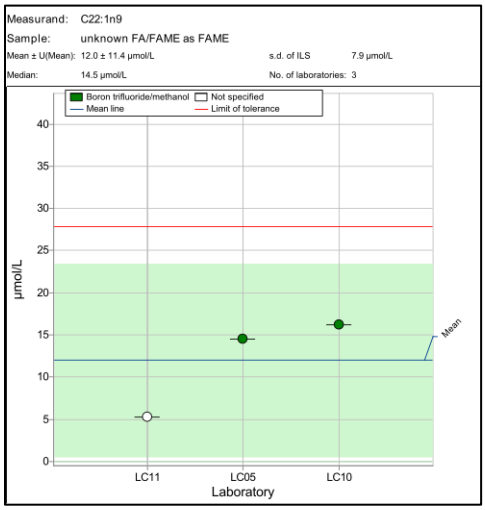
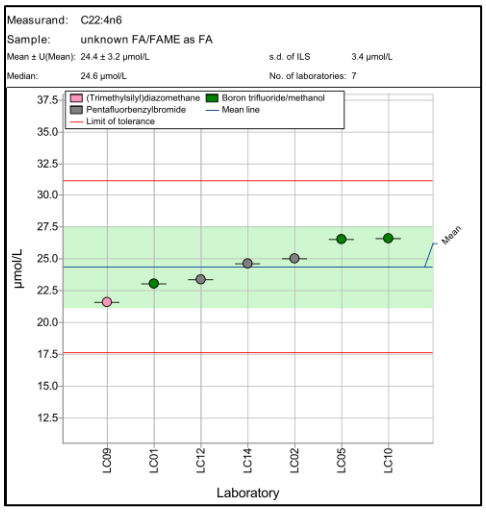


Table B-26: C22:4n6 Docosatetraenoic acid, µmol/L

	FFA Solutions					FA/FAME Solution D			
Lab	A	B	C	Avg	SD	As FA		As FAME	
LC01						23.0			
LC02						25.0			
LC05						26.6		26.6	
LC09						21.6			
LC10						26.6		25.6	
LC11								24.1	
LC12						23.3			
LC14						24.6			
						Avg	24.4	Avg	25.5
						SD	3.4	SD	2.6
						N	7	N	3



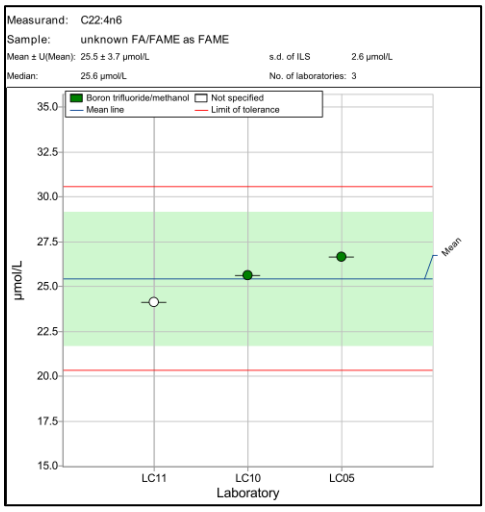


Table B-27: C22:5n3 Docosapentaenoic acid, µmol/L

	FFA Solutions					FA/FAME Solution D			
Lab	A	B	C	Avg	SD	As FA		As FAME	
LC01	33.4	33.6	38.9	35.3	3.1		53.5		
LC02	29.0	30.0	30.0	29.7	0.58		44.0		
LC04	39.5	40.4	41.4	40.5	0.95				
LC05	38.7	38.6	38.5	38.6	0.08		56.1		57.0
LC09	42.1	35.4	35.3	37.6	3.9		54.8		
LC10	36.2	35.1	37.3	36.2	1.1		57.8		54.8
LC11									48.1
LC12	33.4	32.9	33.7	33.3	0.40		49.2		
LC14	37.9	37.4	36.4	37.3	0.8		52.1		
				Avg	36.0	Avg	52.5	Avg	53.3
				SD	4.7	SD	5.8	SD	7.4
				N	8	N	7	N	3

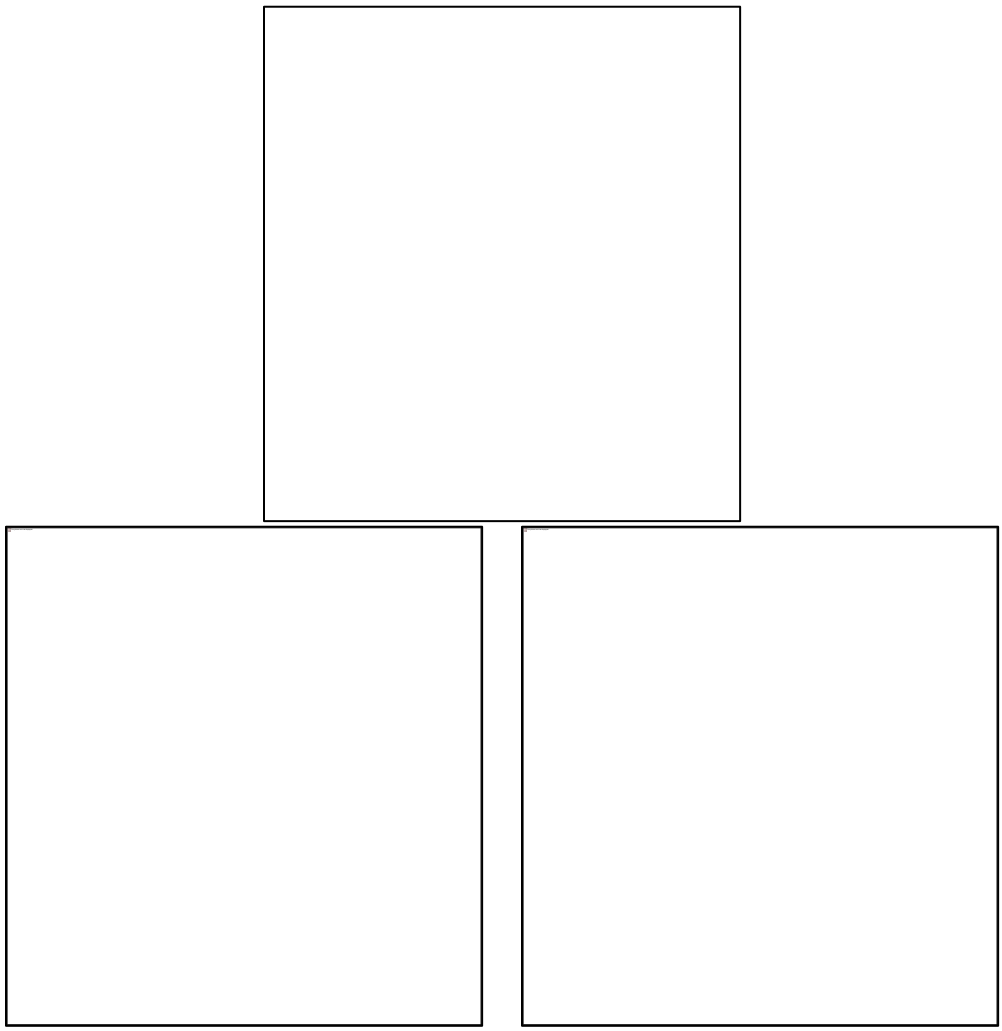
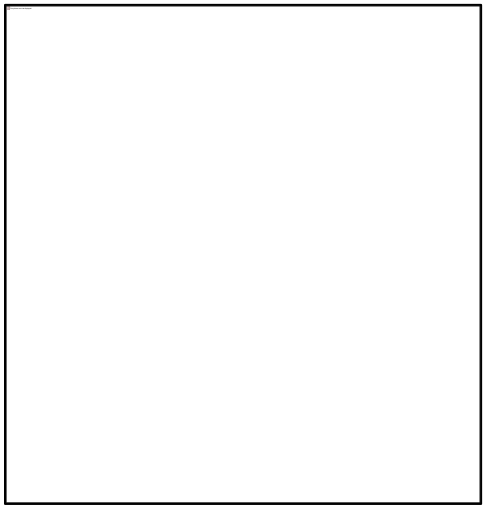


Table B-28: C22:5n6 Docosapentaenoic acid, µmol/L

	FFA Solutions					FA/FAME Solution D			
Lab	A	B	C	Avg	SD	As FA		As FAME	
LC01							25.3		
LC02							25.0		
LC05							27.5		27.8
LC09							22.7		
LC10							26.7		25.6
LC11									10.5
LC12							23.4		
LC14							25.5		
						Avg	25.2	Avg	21.3
						SD	2.4	SD	12
						N	7	N	3



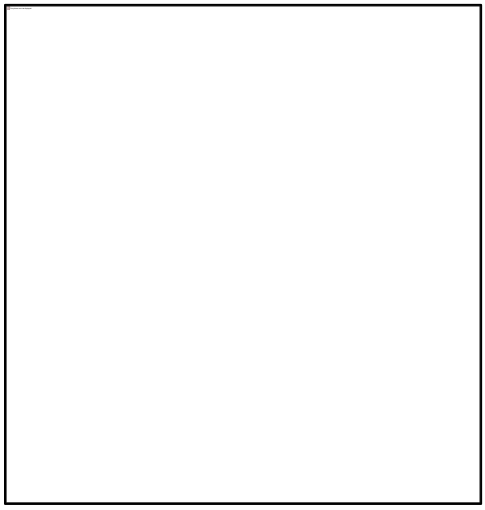
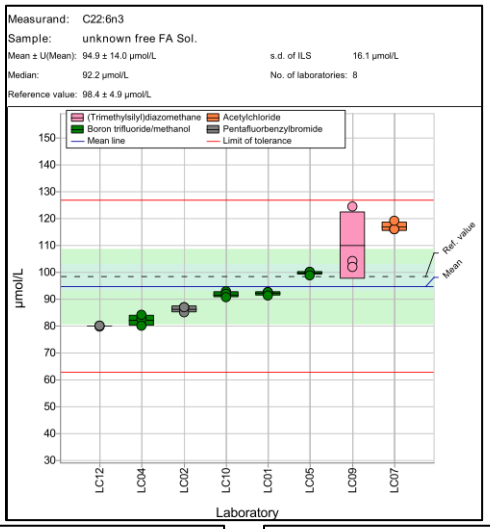
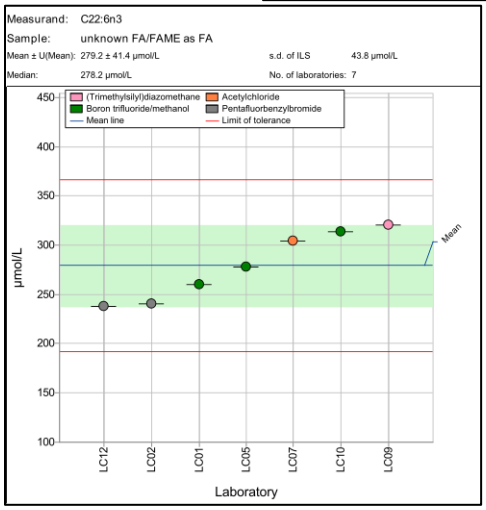


Table B-29: C22:6n3 Docosahexaenoic acid, µmol/L

	FFA Solutions					FA/FAME Solution D			
Lab	A	B	C	Avg	SD	As FA		As FAME	
LC01	92.7	92.6	91.3	92.2	0.77		260		
LC02	87.0	85.0	87.0	86.3	1.15		240		
LC04	82.5	84.0	80.0	82.2	2.0				
LC05	100	100	98.9	99.7	0.69		278		281
LC07	116	116	119	117	1.7		304		341
LC09	125	104	102	110	12		320		
LC10	92.8	91.8	90.7	91.8	1.1		314		301
LC11									319
LC12	79.9	79.8	80.0	79.9	0.10		238		
				Avg	94.9	Avg	279	Avg	310
				SD	16	SD	44	SD	45
				N	8	N	7	N	4





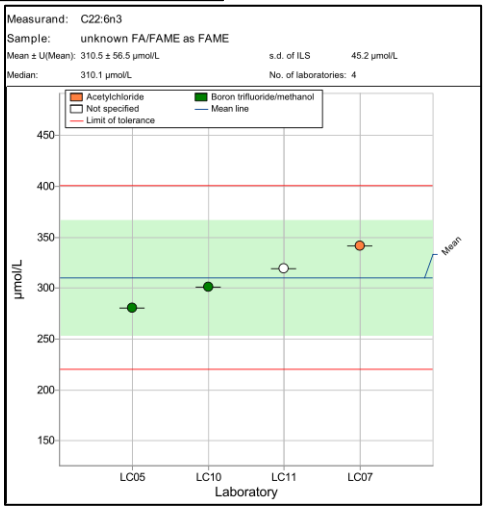
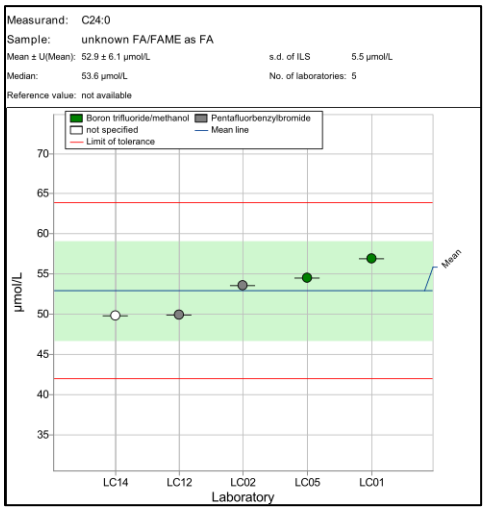


Table B-30: C24:0 Lignoceric acid, µmol/L

	FFA Solutions					FA/FAME Solution D	
Lab	A	B	C	Avg	SD	As FA	As FAME
LC01						56.9	
LC02						53.6	
LC05						54.5	55.5
LC11							24.7
LC12						49.9	
LC14						49.8	
						Avg 52.9	Avg 40.1
						SD 5.5	SD 34
						N 5	N 2



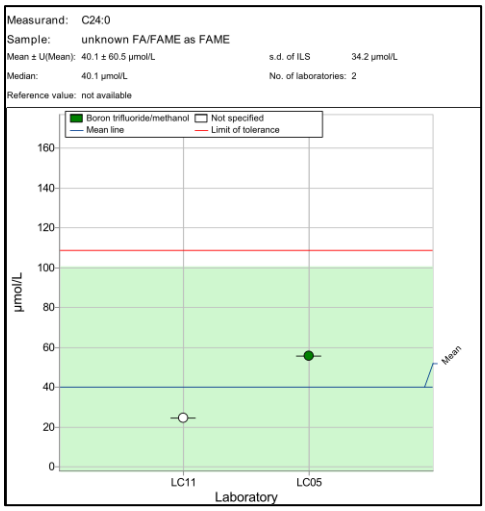
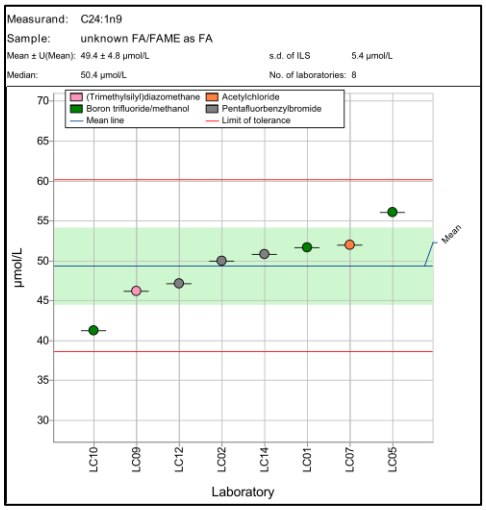
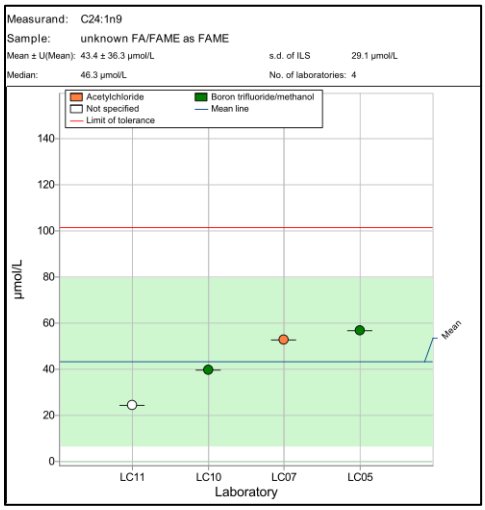


Table B-31: C24:1n9 Nervonic acid, µmol/L

	FFA Solutions					FA/FAME Solution D			
Lab	A	B	C	Avg	SD	As FA		As FAME	
LC01						51.6			
LC02						50.0			
LC05						56.0		56.6	
LC07						52.0		52.8	
LC09						46.2			
LC10						41.2		39.7	
LC11								24.5	
LC12						47.1			
LC14						50.8			
						Avg	49.4	Avg	43.4
						SD	5.4	SD	29
						N	8	N	4





Appendix C: Free Glycerol and Total Glycerides in 2017 Unknown Serum

Acronyms Used in Tables

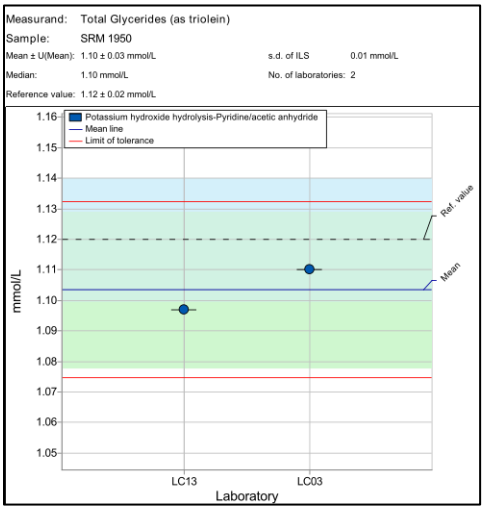
Avg	Mean
Lab	Participant code
N	Number of quantitative results
SD	Standard deviation

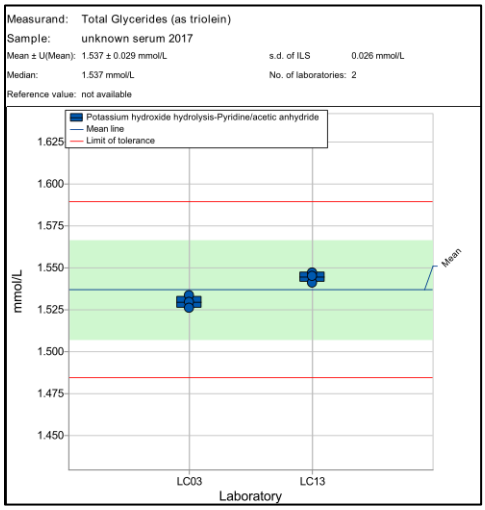
Table C-32: Free Glycerol, mmol/L

		2017 Unknown				
Lab	SRM 1950	A	B	C	Avg	SD
LC13	0.0538	0.0018	0.0016	0.0020	0.00183	0.000

Table C-2: Total Glycerides (as triolein), mmol/L

			2017 Unknown				
Lab	SRM 1950		A	B	C	Avg	SD
NIST	1.12 ± 0.02						
LC03		1.11	1.53	1.53	1.53	1.53	0.0035
LC13		1.10	1.54	1.55	1.55	1.54	0.0031
	Avg	1.10				Avg	1.54
	SD	0.014				SD	0.03
	N	2				N	2





Appendix D: Method Descriptions

List of Tables

Table D-1: Method Description for LC01- Total FA Method

	DESCRIPTION OF PROCEDURES USED FOR TOTAL (CONJUGATED AND FREE) FATTY ACIDS (if evaluated):						
Sample preparation	Volume of sample extracted (mL):		A	B	C	D	
	Weights of samples extracted (g):	Unknown Serum 2017 Samples					
		SRM 1950, control					
Sample processing	Hydrolysis method	14% BF3 in Methanol/Toluene/Methanol heat 100C 45 min					
	Extraction method	LLE					
	Extraction solvent	Hexane					
	Extraction time	60 seconds					
	Extraction - other details	add water too					
	Sample extract cleanup method						
	Derivatization reagent	14% BF3 in MeOH					
Instrumental method	Analytical instrument	GC					
	Column phase	Non-bonded; poly(biscyanopropyl siloxane) (SP-2560)					
	Column length, m	100.00					
	Column i.d., mm	0..25					
	Column film thickness, µm	0.20					
	Injection method (split, splitless, etc.)	Split					
Quantitation	Quant Method: ES = external standards (Y/N)	Yes					
	Number of ES used	3 point standard curve					
	Quant Method: IS = internal standards (Y/N)	Yes					
	Number of IS used	1.00					
	IS used	C23:0 TG					
	IS added PRIOR to extraction of sample (Y/N)	Yes					
	Calibration model (linear, quad)	Linear					
	Calibration range	different for each FA					
Misc.	Additional information, method reference						

Table D-2: Method Description for LC01-Free FA Method

	DESCRIPTION OF PROCEDURES USED FOR FREE FATTY ACIDS (if evaluated):						
Sample preparation	Volume of sample extracted (mL):		A	B	C	D	
	Weights of samples extracted (g):	Unknown Serum 2017 Samples					
		SRM 1950, control					
Sample processing	Hydrolysis method (if applies)	14% BF3 in Methanol/hexane cold temperature					
	Extraction method	Double LLE					
	Extraction solvent	Hexane/Hexane					
	Extraction time						
	Extraction - other details						
	Sample extract cleanup method						
	Derivatization reagent	14% BF3 in MeOH					
Instrumental method	Analytical instrument	GC					
	Column phase	Non-bonded; poly(biscyanopropyl siloxane) (SP-2560)					
	Column length, m	4/9/1900					
	Column i.d., mm	0..25					
	Column film thickness, µm	0.20					
	Injection method (split, splitless, etc.)	Split					
Quantitation	Quant Method: ES = external standards (Y/N)	Yes					
	Number of ES used	3 point standard curve					
	Quant Method: IS = internal standards (Y/N)	Yes					
	Number of IS used	1.00					
	IS used	C23:0 FFA					
	IS added PRIOR to extraction of sample (Y/N)	Yes					
	Calibration model (linear, quad)	Linear					
	Calibration range	different for each FA					
Misc.	Additional information, method reference						

Table D-3: Method Description for LC01-Free FA/FAME in Solutions Method

	DESCRIPTION OF PROCEDURES USED FOR FREE FATTY ACID/FATTY ACID METHYL ESTER SOLUTIONS:						
Sample preparation	Volume of sample extracted (mL):		Unknown Free FA Sol. A	Unknown Free FA Sol. B	Unknown Free FA Sol. C	Unknown FAME/FA Sol. D	
	Weights of samples extracted (g):	Solutions					
Sample processing	Hydrolysis method (if applies)	14% BF3 in Methanol/Toluene/Methanol - heat 100C 45 min					
	Extraction method	LLE					
	Extraction solvent	Hexane					
	Extraction time	60 seconds					
	Extraction - other details	add water too					
	Sample extract cleanup method						
	Derivatization reagent	14% BF3 in MeOH					
Instrumental method	Analytical instrument	GC					
	Column phase	Non-bonded; poly(biscyanopropyl siloxane) (SP-2560)					
	Column length, m	100.00					
	Column i.d., mm	0..25					
	Column film thickness, µm	0.20					
	Injection method (split, splitless, etc.)	Split					
Quantitation	Quant Method: ES = external standards (Y/N)	Yes					
	Number of ES used	3 point standard curve					
	Quant Method: IS = internal standards (Y/N)	Yes					
	Number of IS used	1.00					
	IS used	C23:0 TG					
	IS added PRIOR to extraction of sample (Y/N)	Yes					
	Calibration model (linear, quad)	Linear					
	Calibration range	different for each FA					
Misc.	Additional information, method reference						

Table D-4: Method Description for LC02-Total FA Method

	DESCRIPTION OF PROCEDURES USED FOR TOTAL (CONJUGATED AND FREE) FATTY ACIDS (if evaluated):						
Sample preparation	Volume of sample extracted (mL):	0.05	A	B	C	D	
	Weights of samples extracted (g):	Unknown Serum 2017 Samples	NA	NA	NA		
		SRM 1950, control	NA				
Sample processing	Hydrolysis method	Acid and Base Hydrolysis					
	Extraction method	Liquid Liquid Extraction (LLE)					
	Extraction solvent	Hexane					
	Extraction time	2 minutes					
	Extraction - other details						
	Sample extract cleanup method	Hexane LLE from derivatizing reagent					
	Derivatization reagent	pentafluorobenzylbromide					
Instrumental method	Analytical instrument	6890N/5973N Gas Chromatograph/Mass Spectrometer					
	Column phase	5% phenyl-95% methylpolysiloxane					
	Column length, m	20m					
	Column i.d., mm	0.18					
	Column film thickness, µm	180.00					
	Injection method (split, splitless, etc.)	split and splitless					
Quantitation	Quant Method: ES = external standards (Y/N)	N					
	Number of ES used	0.00					
	Quant Method: IS = internal standards (Y/N)	Y					
	Number of IS used	13.00					
	IS used	Y					
	IS added PRIOR to extraction of sample (Y/N)	Y					
	Calibration model (linear, quad)	Linear					
	Calibration range	Varies by fatty acid					
Misc	Additional information, method reference	Lagerstedt SA, Hinrichs DR, Batt SM, Magera MJ, Rinaldo P, McConnell JP. (2001) Quantitative determination of plasma C8-C26 total fatty acids for the biochemical diagnosis of nutritional and metabolic disorders. Mol Genet Metab,.73:38-45.					

Table D-5: Method Description for LC02-Free FA/FAME in Solutions Method

	DESCRIPTION OF PROCEDURES USED FOR FREE FATTY ACID/FATTY ACID METHYL ESTER SOLUTIONS:						
Sample preparation	Volume of sample extracted (mL):	0.05	Unknown Free FA Sol. A	Unknown Free FA Sol. B	Unknown Free FA Sol. C	Unknown FAME/FA Sol. D	
	Weights of samples extracted (g):	Solutions	NA	NA	NA	NA	
Sample processing	Hydrolysis method (if applies)	Acid and Base Hydrolysis					
	Extraction method	Liquid Liquid Extraction (LLE)					
	Extraction solvent	Hexane					
	Extraction time	2 minutes					
	Extraction - other details						
	Sample extract cleanup method	Hexane LLE from derivatizing reagent					
	Derivatization reagent	pentafluorobenzylbromide					
Instrumental method	Analytical instrument	6890N/5973N Gas Chromatograph/Mass Spectrometer					
	Column phase	5% phenyl-95% methylpolysiloxane					
	Column length, m	20m					
	Column i.d., mm	0.18					
	Column film thickness, µm	180.00					
	Injection method (split, splitless, etc.)	split and splitless					
Quantitation	Quant Method: ES = external standards (Y/N)	N					
	Number of ES used	0.00					
	Quant Method: IS = internal standards (Y/N)	Y					
	Number of IS used	13.00					
	IS used	Y					
	IS added PRIOR to extraction of sample (Y/N)	Y					
	Calibration model (linear, quad)	Linear					
	Calibration range	Varies by fatty acid					
Misc.	Additional information, method reference	Lagerstedt SA, Hinrichs DR, Batt SM, Magera MJ, Rinaldo P, McConnell JP. (2001) Quantitative determination of plasma C8-C26 total fatty acids for the biochemical diagnosis of nutritional and metabolic disorders. Mol Genet Metab,.73:38-45.					

Table D-6: Method Descriptions for LC03-Total Glycerides Method

	DESCRIPTION OF PROCEDURES USED FOR TOTAL GLYCERIDES (if evaluated):						
Sample preparation	Volume of sample extracted (mL):	0.8 ml	A	B	C	D	
	Weights of samples extracted (g):	Unknown Serum 2017 Samples	0.8245	0.8251	0.8221		
		SRM 1950, control	0.8219				
Sample processing	Hydrolysis method (if applies)	Ethanol/KOH solution for 1h, 70C					
	Extraction method	liquid-liquid					
	Extraction solvent	First extraction: ethyl acetate/water					
	Extraction time	15 min					
	Extraction - other details						
	Sample extract cleanup method	Followed by: 2 x sodium bicarbonate and 1 x di water clean up steps					
	Derivatization reagent	acetic anhydride in pyridine					
Instrumental method	Analytical instrument	GC-MS					
	Column phase	Zebron ZB-50					
	Column length, m	30					
	Column i.d., mm	0.25					
	Column film thickness, µm	0.25					
	Injection method (split, splitless, etc.)	splitless					
Quantitation	Quant Method: ES = external standards (Y/N)	N					
	Number of ES used	NA					
	Quant Method: IS = internal standards (Y/N)	Y					
	Number of IS used	1					
	IS used	[13C3]-glycerol					
	IS added PRIOR to extraction of sample (Y/N)	Y					
	Calibration model (linear, quad)	linear					
	Calibration range	0.056-1.129 mmol/L (samples are diluted x3; therefore the actual measurement range is 0.169-3.388 mmol/L)					
Misc.	Additional information, method reference	Reference Measurement Procedure for Total Glycerides by Isotope Dilution GC-MS, (JCTLM C12RMP5) Edwards S. et al., Clinical Chemistry, 2012, 58(4), 768-776					

Table D-7: Method Descriptions for LC04-Total FA Method

	DESCRIPTION OF PROCEDURES USED FOR TOTAL (CONJUGATED AND FREE) FATTY ACIDS (if evaluated):						
Sample preparation	Volume of sample extracted (mL):	0.100	A	B	C	D	
	Weights of samples extracted (g):	Unknown Serum 2017 Samples					
		SRM 1950, control					
Sample processing	Hydrolysis method	transmethylation with hexane + BF3/methanol (14%, g/L)					
	Extraction method	Folch					
	Extraction solvent	chloroform : methanol : buffer					
	Extraction time	twice					
	Extraction - other details						
	Sample extract cleanup method	water was applied after transmethylation					
	Derivatization reagent	BF3/methanol (14%, g/L)					
Instrumental method	Analytical instrument	7890A GC/FID					
	Column phase	DB-FFAP					
	Column length, m	15					
	Column i.d., mm	0.10					
	Column film thickness, µm	0.10					
	Injection method (split, splitless, etc.)	split					
Quantitation	Quant Method: ES = external standards (Y/N)	Y					
	Number of ES used	28					
	Quant Method: IS = internal standards (Y/N)	Y					
	Number of IS used	1					
	IS used	22:3n-3					
	IS added PRIOR to extraction of sample (Y/N)	Y					
	Calibration model (linear, quad)	linear					
	Calibration range	1 - 600 µg/mL					
Misc.	Additional information, method reference						

Table D-8: Method Descriptions for LC04-Free FA/FAME in Solutions Method

	DESCRIPTION OF PROCEDURES USED FOR FREE FATTY ACID/FATTY ACID METHYL ESTER SOLUTIONS:						
Sample preparation	Volume of sample extracted (mL):	0.100	Unknown Free FA Sol. A	Unknown Free FA Sol. B	Unknown Free FA Sol. C	Unknown FAME/FA Sol. D	
	Weights of samples extracted (g):	Solutions					
Sample processing	Hydrolysis method (if applies)	N/A					
	Extraction method	N/A					
	Extraction solvent						
	Extraction time						
	Extraction - other details						
	Sample extract cleanup method						
	Derivatization reagent	BF3/methanol (14%, g/L)					
Instrumental method	Analytical instrument	same as above					
	Column phase						
	Column length, m						
	Column i.d., mm						
	Column film thickness, µm						
	Injection method (split, splitless, etc.)						
Quantitation	Quant Method: ES = external standards (Y/N)	same as above					
	Number of ES used						
	Quant Method: IS = internal standards (Y/N)						
	Number of IS used						
	IS used						
	IS added PRIOR to extraction of sample (Y/N)						
	Calibration model (linear, quad)						
	Calibration range						
Misc.	Additional information, method reference						

Table D-9: Method Descriptions for LC05-Total FA Method

	DESCRIPTION OF PROCEDURES USED FOR TOTAL (CONJUGATED AND FREE) FATTY ACIDS (if evaluated):						
Sample preparation	Volume of sample extracted (mL):	0.10	A	B	C	D	
	Weights of samples extracted (g):	Unknown Serum 2017 Samples	0.106	0.106	0.106		
		SRM 1950, control	0.107				
Sample processing	Hydrolysis method	NA, direct transesterification of lipid extracts with methylating reagents					
	Extraction method	Modified Folch					
	Extraction solvent	3mL of 2:1:0.5 chloroform:methanol:0.2M sodium phosphate buffer, C22:3n-3 free fatty acid internal standard					
	Extraction time	30 minutes					
	Extraction - other details	NA					
	Sample extract cleanup method	FAME cleanup with 1mL ddH2O and 1mL hexane					
	Derivatization reagent	14% boron trifluoride in methanol					
Instrumental method	Analytical instrument	Varian 3900 Gas Chromatograph					
	Column phase	DB-FFAP nitroterephthalic acid modified polyethylene glycol capillary column					
	Column length, m	15.00					
	Column i.d., mm	0.10					
	Column film thickness, µm	0.10					
	Injection method (split, splitless, etc.)	Split injection, 1:100					
Quantitation	Quant Method: ES = external standards (Y/N)	Y					
	Number of ES used	39.00					
	Quant Method: IS = internal standards (Y/N)	Y					
	Number of IS used	1.00					
	IS used	C 22:3n-3 free fatty acid					
	IS added PRIOR to extraction of sample (Y/N)	Y					
	Calibration model (linear, quad)	Linear					
	Calibration range	0.0016ug/mL solvent - 75 ug/mL solvent for each fatty acid, using GLC-462					
Misc.	Additional information, method reference						

Table D-10: Method Descriptions for LC05-Free FA Method

	DESCRIPTION OF PROCEDURES USED FOR FREE FATTY ACIDS (if evaluated):						
Sample preparation	Volume of sample extracted (mL):	0.10	A	B	C	D	
	Weights of samples extracted (g):	Unknown Serum 2017 Samples	0.107	0.106	0.107		
		SRM 1950, control	0.107				
Sample processing	Hydrolysis method (if applies)	NA					
	Extraction method	Modified Folch					
	Extraction solvent	3mL of 2:1:0.5 chloroform:methanol:0.2M sodium phosphate buffer, C22:3n-3 free fatty acid internal standard					
	Extraction time	30 minutes					
	Extraction - other details	NA					
	Sample extract cleanup method	FAME cleanup with 1mL ddH2O and 1mL hexane					
	Derivatization reagent	14% boron trifluoride in methanol after FFA separation by thin layer chromatography and extraction using chloroform/methanol					
Instrumental method	Analytical instrument	Varian 3900 Gas Chromatograph					
	Column phase	DB-FFAP nitroterephthalic acid modified polyethylene glycol capillary column					
	Column length, m	15.00					
	Column i.d., mm	0.10					
	Column film thickness, µm	0.10					
	Injection method (split, splitless, etc.)	Split injection, 1:20					
Quantitation	Quant Method: ES = external standards (Y/N)	Y					
	Number of ES used	39.00					
	Quant Method: IS = internal standards (Y/N)	Y					
	Number of IS used	1.00					
	IS used	C 22:3n-3 free fatty acid					
	IS added PRIOR to extraction of sample (Y/N)	Y					
	Calibration model (linear, quad)	Linear					
	Calibration range	0.0016ug/mL solvent - 75 ug/mL solvent for each fatty acid, using GLC-462					
Misc.	Additional information, method reference	Thin layer chromatography was performed using a mobile phase consisting of 60:40:2 heptane:anhydrous diethyl ether:glacial acetic acid, used a mixture of external reference standards for visualization under UV light, samples were scraped and lipids were extracted from silica shavings using 3mL 2:1 chloroform:methanol. Free fatty acid extracts were then methylated and analyzed by GC.					

Table D-11: Method Descriptions for LC05-Free FA/FAME in Solutions Method

	DESCRIPTION OF PROCEDURES USED FOR FREE FATTY ACID/FATTY ACID METHYL ESTER SOLUTIONS:						
Sample preparation	Volume of sample extracted (mL):	0.10	Unknown Free FA Sol. A	Unknown Free FA Sol. B	Unknown Free FA Sol. C	Unknown FAME/FA Sol. D	
	Weights of samples extracted (g):	Solutions	100uL	100uL	100uL	100uL	
Sample processing	Hydrolysis method (if applies)	NA					
	Extraction method	NA, FFA and FAME solutions were derivatized to generate FAME after aliquoting samples and evaporating toluene under N2 gas					
	Extraction solvent	NA					
	Extraction time	NA					
	Extraction - other details	NA					
	Sample extract cleanup method	FAME cleanup with 1mL ddH2O and 1mL hexane					
	Derivatization reagent	14% boron trifluoride in methanol					
Instrumental method	Analytical instrument	Varian 3900 Gas Chromatograph					
	Column phase	DB-FFAP nitroterephthalic acid modified polyethylene glycol capillary column					
	Column length, m	15.00					
	Column i.d., mm	0.10					
	Column film thickness, µm	0.10					
	Injection method (split, splitless, etc.)	Split injection, 1:100					
Quantitation	Quant Method: ES = external standards (Y/N)	Y					
	Number of ES used	39.00					
	Quant Method: IS = internal standards (Y/N)	Y					
	Number of IS used	1.00					
	IS used	C 22:3n-3 free fatty acid					
	IS added PRIOR to extraction of sample (Y/N)	Y					
	Calibration model (linear, quad)	Linear					
	Calibration range	0.0016ug/mL solvent - 75 ug/mL solvent for each fatty acid, using GLC-462					
Misc.	Additional information, method reference	Samples were not weighed to determine density because of high volatility of sample solvent, scale did not stabilize					

Table D-12: Method Descriptions for LC06-Free FA Method

	DESCRIPTION OF PROCEDURES USED FOR FREE FATTY ACIDS (if evaluated):						
Sample preparation	Volume of sample extracted (mL):		A	B	C	D	
	Weights of samples extracted (g):	Unknown Serum 2017 Samples	0.25268	0.25571	0.25745		
		SRM 1950, control	0.25				
Sample processing	Hydrolysis method (if applies)	None					
	Extraction method	2, 2 mL, liquid/liquid extractions.					
	Extraction solvent	ethyl acetate					
	Extraction time	1 min vortex					
	Extraction - other details						
	Sample extract cleanup method	none					
	Derivatization reagent	none					
Instrumental method	Analytical instrument	Agilent 5975C					
	Column phase	ZB-FFAP					
	Column length, m	60					
	Column i.d., mm	0.25					
	Column film thickness, µm	0.25					
	Injection method (split, splitless, etc.)	on-column					
Quantitation	Quant Method: ES = external standards (Y/N)	N					
	Number of ES used	NA					
	Quant Method: IS = internal standards (Y/N)	Y					
	Number of IS used	3.00					
	IS used	myristic-d27, palmitic-d31, and steric-d35					
	IS added PRIOR to extraction of sample (Y/N)	Y					
	Calibration model (linear, quad)	single point					
	Calibration range						
Misc.	Additional information, method reference						

Table D-13: Method Descriptions for LC06-Free FA/FAME in Solutions Method

	DESCRIPTION OF PROCEDURES USED FOR FREE FATTY ACID/FATTY ACID METHYL ESTER SOLUTIONS:						
Sample preparation	Volume of sample extracted (mL):		Unknown Free FA Sol. A	Unknown Free FA Sol. B	Unknown Free FA Sol. C	Unknown FAME/FA Sol. D	
	Weights of samples extracted (g):	Solutions	0.04104	0.04193	0.04235		
Sample processing	Hydrolysis method (if applies)	None					
	Extraction method	None					
	Extraction solvent						
	Extraction time						
	Extraction - other details						
	Sample extract cleanup method	none					
	Derivatization reagent	none					
Instrumental method	Analytical instrument						
	Column phase	ZB-FFAP					
	Column length, m	60					
	Column i.d., mm	0.25					
	Column film thickness, µm	0.25					
	Injection method (split, splitless, etc.)	on-column					
Quantitation	Quant Method: ES = external standards (Y/N)	N					
	Number of ES used	NA					
	Quant Method: IS = internal standards (Y/N)	Y					
	Number of IS used	3.00					
	IS used	myristic-d27, palmitic-d31, and steric-d35					
	IS added PRIOR to extraction of sample (Y/N)	Y					
	Calibration model (linear, quad)	single point					
	Calibration range						
Misc.	Additional information, method reference						

Table D-14: Method Descriptions for LC07: Total FA Method

	DESCRIPTION OF PROCEDURES USED FOR TOTAL (CONJUGATED AND FREE) FATTY ACIDS (if evaluated):						
Sample preparation	Volume of sample extracted (mL):	0.1	A	B	C	D	
	Weights of samples extracted (g):	Unknown Serum 2017 Samples					
		SRM 1950, control					
Sample processing	Hydrolysis method (if applies)						
	Extraction method	FAME, methanol					
	Extraction solvent	Toluene					
	Extraction time	2 h					
	Extraction - other details	80 °C, sealed tubes, quench with potassium carbonate, vortex, centrifugation 10 min					
	Sample extract cleanup method						
	Derivatization reagent	Acetylchloride					
Instrumental method	Analytical instrument	Shimadzu GC 2010 plus					
	Column phase	Agilent J&W HP-88					
	Column length, m	100 m					
	Column i.d., mm	0.25					
	Column film thickness, µm	0.20					
	Injection method (split, splitless, etc.)	1:10 split					
Quantitation	Quant Method: ES = external standards (Y/N)	Y					
	Number of ES used	4.00					
	Quant Method: IS = internal standards (Y/N)	Y					
	Number of IS used	3.00					
	IS used	C170, C190, C270					
	IS added PRIOR to extraction of sample (Y/N)	Y					
	Calibration model (linear, quad)	linear through zero					
	Calibration range	blank to approx. 1600 mg/L					
Misc.	Additional information, method reference						

Table D-15: Method Descriptions for LC07: Free FA/FAME in Solutions Method

	DESCRIPTION OF PROCEDURES USED FOR FREE FATTY ACID/FATTY ACID METHYL ESTER SOLUTIONS:						
Sample preparation	Volume of sample extracted (mL):	0.10	Unknown Free FA Sol. A	Unknown Free FA Sol. B	Unknown Free FA Sol. C	Unknown FAME/FA Sol. D	
	Weights of samples extracted (g):	Solutions					
Sample processing	Hydrolysis method (if applies)						
	Extraction method	FAME, methanol					
	Extraction solvent	Toluene					
	Extraction time	2 h					
	Extraction - other details	80 °C, sealed tubes, quench with potassium carbonate, vortex, centrifugation 10 min					
	Sample extract cleanup method						
	Derivatization reagent	Acetylchloride					
Instrumental method	Analytical instrument	Shimadzu GC 2010 plus					
	Column phase	Agilent J&W HP-88					
	Column length, m	100 m					
	Column i.d., mm	0.25					
	Column film thickness, µm	0.20					
	Injection method (split, splitless, etc.)	1:10 split					
Quantitation	Quant Method: ES = external standards (Y/N)	Y					
	Number of ES used	4.00					
	Quant Method: IS = internal standards (Y/N)	Y					
	Number of IS used	3.00					
	IS used	C170, C190, C270					
	IS added PRIOR to extraction of sample (Y/N)	Y					
	Calibration model (linear, quad)	linear through zero					
	Calibration range	blank to approx. 1600 mg/L					
Misc.	Additional information, method reference	We spike our standards into BSA/NaCl ("fake" serum). Therefore, density is not a variable as this "fake" serum has a density of 1,02. Even though the standards are already FAMEs, they are subjected to a complete sample workup.					

Table D-16: Method Descriptions for LC08-Total FA Method

	DESCRIPTION OF PROCEDURES USED FOR TOTAL (CONJUGATED AND FREE) FATTY ACIDS (if evaluated):						
Sample preparation	Volume of sample extracted (mL):	0.100	A	B	C	D	
	Weights of samples extracted (g):	Unknown Serum 2017 Samples					
		SRM 1950, control					
Sample processing	Hydrolysis method	5% v/v hydrochrloric methanol, 90oC-60min					
	Extraction method	solvent extraction with hexane after 1 min vortex					
	Extraction solvent	hexane					
	Extraction time	1 min vortex					
	Extraction - other details	the fatty acids were axtracted with hexane as methyl esters after the methylation procedure					
	Sample extract cleanup method	centrifugation, 8000rpm, 10 min					
	Derivatization reagent	5% v/v hydrochrloric methanol, 90oC-60min					
Instrumental method	Analytical instrument	Agilent 7890A/5975C GC/MS operating in electron ionization mode					
	Column phase	HP-5ms capillary column					
	Column length, m	30.00					
	Column i.d., mm	0.25					
	Column film thickness, µm	0.25					
	Injection method (split, splitless, etc.)	splitless					
Quantitation	Quant Method: ES = external standards (Y/N)	N					
	Number of ES used						
	Quant Method: IS = internal standards (Y/N)	Y					
	Number of IS used	1.00					
	IS used	methyl nonadecanoate					
	IS added PRIOR to extraction of sample (Y/N)	Y					
	Calibration model (linear, quad)	linear					
	Calibration range	5-5000 µmol/L					
Misc	Additional information, method reference	The analysis of Total Fatty Acids involves hydrolysis of the sample and simultaneous formation of the fatty acid methyl esters with the addition of hydrochloric methanol and incubation for 60 min at 90oC .					

Table D-17: Method Descriptions for LC08-Free FA/FAME in Solutions Method

	DESCRIPTION OF PROCEDURES USED FOR FREE FATTY ACID/FATTY ACID METHYL ESTER SOLUTIONS:						
Sample preparation	Volume of sample extracted (mL):	0.100	Unknown Free FA Sol. A	Unknown Free FA Sol. B	Unknown Free FA Sol. C	Unknown FAME/FA Sol. D	
	Weights of samples extracted (g):	Solutions					
Sample processing	Hydrolysis method (if applies)	5% v/v hydrochrloric methanol, 90oC-60min					
	Extraction method	solvent extraction with hexane after 1 min vortex					
	Extraction solvent	hexane					
	Extraction time	1 min vortex					
	Extraction - other details	the fatty acids were axtracted with hexane as methyl esters after the methylation procedure					
	Sample extract cleanup method	centrifugation, 8000rpm, 10 min					
	Derivatization reagent	5% v/v hydrochrloric methanol, 90oC-60min					
Instrumental method	Analytical instrument	Agilent 7890A/5975C GC/MS operating in electron ionization mode					
	Column phase	HP-5ms capillary column					
	Column length, m	30.00					
	Column i.d., mm	0.25					
	Column film thickness, µm	0.25					
	Injection method (split, splitless, etc.)	splitless					
Quantitation	Quant Method: ES = external standards (Y/N)	N					
	Number of ES used						
	Quant Method: IS = internal standards (Y/N)	Y					
	Number of IS used	1.00					
	IS used	methyl nonadecanoate					
	IS added PRIOR to extraction of sample (Y/N)	Y					
	Calibration model (linear, quad)	linear					
	Calibration range	5-5000 µmol/L					
Misc.	Additional information, method reference	We measured the free FA in the three solutions in the Fatty Acid methyl ester form.					

Table D-18: Method Descriptions for LC09-Total FA Method

	DESCRIPTION OF PROCEDURES USED FOR TOTAL (CONJUGATED AND FREE) FATTY ACIDS (if evaluated):						
Sample preparation	Volume of sample extracted (mL):		A	B	C	D	
	Weights of samples extracted (g):	Unknown Serum 2017 Samples	50µL	50µL	50µL		
		SRM 1950, control	50µL				
Sample processing	Hydrolysis method	0.5M dry methanolic sodium methoxide (as part of derivatization)					
	Extraction method	Based on F. Smedes, Analyst, 1999,124, 1711-1718					
	Extraction solvent	50µL plasma extracted with 0.41mL 2-propanol, 0.52mL cyclohexane, 0.57mL 0.1M ammonium acetate, 0.52mL cyclohexane					
	Extraction time	6 minutes					
	Extraction - other details	Transfer cyclohexane fraction and re-extract with added 0.52mL cyclohexane					
	Sample extract cleanup method	Evaporate to dryness and reconstitute in 200µL 1:1 methanol/toluene, use 40µL aliquot for total fatty acids plus 160µL methanol					
	Derivatization reagent	0.5M dry methanolic sodium methoxide, 60oC 60 min, +0.1mL 3N Methanolic HCl, 60oC 30 min, neutralize, extract with 0.4mL hexanes					
Instrumental method	Analytical instrument	Agilent 6890 Plus+ GC, 5973N MSD, 7683 Injector, DB-225ms column					
	Column phase	(50%-Cyanopropylphenyl)-dimethylpolysiloxane					
	Column length, m	30.00					
	Column i.d., mm	0.25					
	Column film thickness, µm	0.25					
	Injection method (split, splitless, etc.)	splitless					
Quantitation	Quant Method: ES = external standards (Y/N)	N					
	Number of ES used	0.00					
	Quant Method: IS = internal standards (Y/N)	Y					
	Number of IS used	4 Surrogates at start of extraction, 1 surrogate at start of derivatization, 1 Internal Standard before analysis					
	IS used	(TAG-16:0-d31, PC-18:0-d35, CE-22:1n9, C22:3n3) at start of extraction, C15:1n5 at start of derivatization, C23:0 Internal Standard before analysis					
	IS added PRIOR to extraction of sample (Y/N)	N					
	Calibration model (linear, quad)	quadratic					
	Calibration range	1 nM to 200 µM					
Misc.	Additional information, method reference	Blanks were subtracted on a per sample basis by multiplying the amount of C10:0 in the sample by the analyte to C10:0 ratio in the method blanks. Analytes were not reported when the method blank value was more than 60% of the samples average.					

Table D-19: Method Descriptions for LC09-Free FA Method

	DESCRIPTION OF PROCEDURES USED FOR FREE FATTY ACIDS (if evaluated):						
Sample preparation	Volume of sample extracted (mL):		A	B	C	D	
	Weights of samples extracted (g):	Unknown Serum 2017 Samples	50µL	50µL	50µL		
		SRM 1950, control	50µL				
Sample processing	Hydrolysis method (if applies)	none					
	Extraction method	Based on F. Smedes, Analyst, 1999,124, 1711-1718					
	Extraction solvent	50µL plasma extracted with 0.41mL 2-propanol, 0.52mL cyclohexane, 0.57mL 0.1M ammonium acetate, 0.52mL cyclohexane					
	Extraction time	6 minutes					
	Extraction - other details	Transfer cyclohexane fraction and re-extract with added 0.52mL cyclohexane					
	Sample extract cleanup method	Evaporate to dryness and reconstitute in 200µL 1:1 methanol/toluene, use 160µL aliquot for total fatty acids plus 120µL methanol & 20µL toluene					
	Derivatization reagent	45µL (trimethylsilyl)diazomethane (in nhexanes) for 30 minutes, evaporate to dryness, reconstitute in 200µL hexanes					
Instrumental method	Analytical instrument	Agilent 6890 Plus+ GC, 5973N MSD, 7683 Injector, DB-225ms column					
	Column phase	(50%-Cyanopropylphenyl)-dimethylpolysiloxane					
	Column length, m	30.00					
	Column i.d., mm	0.25					
	Column film thickness, µm	0.25					
	Injection method (split, splitless, etc.)	splitless					
Quantitation	Quant Method: ES = external standards (Y/N)	N					
	Number of ES used	0.00					
	Quant Method: IS = internal standards (Y/N)	Y					
	Number of IS used	4 Surrogates at start of extraction, 1 surrogate at start of derivatization, 1 Internal Standard before analysis					
	IS used	(TAG-16:0-d31, PC-18:0-d35, CE-22:1n9, C22:3n3) at start of extraction, C15:1n5 at start of derivatization, C23:0 Internal Standard before analysis					
	IS added PRIOR to extraction of sample (Y/N)	N					
	Calibration model (linear, quad)	quadratic					
	Calibration range	1 nM to 200 µM					
Misc.	Additional information, method reference	Blanks were subtracted on a per sample basis by multiplying the amount of C10:0 in the sample by the analyte to C10:0 ratio in the method blanks. Analytes were not reported when the method blank value was more than 60% of the samples average.					

Table D-20: Method Descriptions for LC09-Free FA/FAME in Solutions Method

	DESCRIPTION OF PROCEDURES USED FOR FREE FATTY ACID/FATTY ACID METHYL ESTER SOLUTIONS:						
Sample preparation	Volume of sample extracted (mL):		Unknown Free FA Sol. A	Unknown Free FA Sol. B	Unknown Free FA Sol. C	Unknown FAME/FA Sol. D	
	Weights of samples extracted (g):	Solutions	40µL	40µL	40µL	40µL	
Sample processing	Hydrolysis method (if applies)	none					
	Extraction method	none					
	Extraction solvent	none					
	Extraction time	N/A					
	Extraction - other details	N/A					
	Sample extract cleanup method	Dilute 40µL sample (in toluene) with 60µL toluene and 200µL methanol					
	Derivatization reagent	45µL (trimethylsilyl)diazomethane (in nhexanes), derivatize for 30 minutes, evaporate to dryness, reconstitute in 200µL hexanes					
Instrumental method	Analytical instrument	Agilent 6890 Plus+ GC, 5973N MSD, 7683 Injector, DB-225ms column					
	Column phase	(50%-Cyanopropylphenyl)-dimethylpolysiloxane					
	Column length, m	30.00					
	Column i.d., mm	0.25					
	Column film thickness, µm	0.25					
	Injection method (split, splitless, etc.)	splitless					
Quantitation	Quant Method: ES = external standards (Y/N)	N					
	Number of ES used	0.00					
	Quant Method: IS = internal standards (Y/N)	Y					
	Number of IS used	5 Surrogates at start of derivatization, 1 Internal Standard before analysis					
	IS used	(TAG-16:0-d31, PC-18:0-d35, CE-22:1n9, C22:3n3 and C15:1n5) at start of derivatization, C23:0 Internal Standard before analysis					
	IS added PRIOR to extraction of sample (Y/N)	N					
	Calibration model (linear, quad)	quadratic					
	Calibration range	1 nM to 200 µM					
Misc.	Additional information, method reference	Blanks were subtracted on a per sample basis by multiplying the amount of C10:0 in the sample by the analyte to C10:0 ratio in the method blanks. Analytes were not reported when the method blank value was more than 60% of the samples average. The values for C18:0, C18:1n7, and C18:1n9 were above the highest standard in the calibration curve (144%, 142%, and 290% of the top standard respectively). All measurements were done as samples derivatized to FAME's so FAME values could not be determined separately from FAs in the samples.					

Table D-21: Method Descriptions for LC10-Total FA Method

	DESCRIPTION OF PROCEDURES USED FOR TOTAL (CONJUGATED AND FREE) FATTY ACIDS (if evaluated):						
Sample preparation	Volume of sample extracted (mL):	0.05	A	B	C	D	
	Weights of samples extracted (g):	Unknown Serum 2017 Samples	0.051	0.051	0.051		
		SRM 1950, control	0.051				
Sample processing	Hydrolysis method	Alkaline hydrolysis					
	Extraction method	first hexane extract containing neutral lipids is discarded; the acidified aqueous phase is re-extracted with hexane to obtain total fatty acids					
	Extraction solvent	hexane					
	Extraction time	30min					
	Extraction - other details	derivatized fatty acids (FAMEs) are extracted in hexane/saturated NaCl;					
	Sample extract cleanup method	hexane is evaporated under N2 and samples dissolved in iso-octane for GC analysis					
	Derivatization reagent	boron trifluoride in methanol					
Instrumental method	Analytical instrument	Agilent 7890B					
	Column phase	Agilent DB-23					
	Column length, m	30m					
	Column i.d., mm	0.25					
	Column film thickness, µm	0.25					
	Injection method (split, splitless, etc.)	cool on column					
Quantitation	Quant Method: ES = external standards (Y/N)	N					
	Number of ES used						
	Quant Method: IS = internal standards (Y/N)	yes					
	Number of IS used	1.00					
	IS used	triheptadecanoin					
	IS added PRIOR to extraction of sample (Y/N)	yes					
	Calibration model (linear, quad)	linear					
	Calibration range						
Misc.	Additional information, method reference						

Table D-22: Method Descriptions for LC10-Free FA/FAME in Solutions Method

	DESCRIPTION OF PROCEDURES USED FOR FREE FATTY ACID/FATTY ACID METHYL ESTER SOLUTIONS:						
Sample preparation	Volume of sample extracted (mL):	0.05	Unknown Free FA Sol. A	Unknown Free FA Sol. B	Unknown Free FA Sol. C	Unknown FAME/FA Sol. D	
	Weights of samples extracted (g):	Solutions	0.04	0.04	0.04	0.04	
Sample processing	Hydrolysis method (if applies)	Alkaline hydrolysis					
	Extraction method	first hexane extract containing neutral lipids is discarded; the acidified aqueous phase is re-extracted with hexane to obtain total fatty acids					
	Extraction solvent	hexane					
	Extraction time	30min					
	Extraction - other details	derivatized fatty acids (FAMEs) are extracted in hexane/saturated NaCl;					
	Sample extract cleanup method	hexane is evaporated under N2 and samples dissolved in iso-octane for GC analysis					
	Derivatization reagent	boron trifluoride in methanol					
Instrumental method	Analytical instrument	Agilent 7890B					
	Column phase	Agilent DB-23					
	Column length, m	30m					
	Column i.d., mm	0.25					
	Column film thickness, µm	0.25					
	Injection method (split, splitless, etc.)	cool on column					
Quantitation	Quant Method: ES = external standards (Y/N)	N					
	Number of ES used						
	Quant Method: IS = internal standards (Y/N)	yes					
	Number of IS used	1.00					
	IS used	triheptadecanoin					
	IS added PRIOR to extraction of sample (Y/N)	yes					
	Calibration model (linear, quad)	linear					
	Calibration range						
Misc.	Additional information, method reference						

Table D-23: Method Descriptions for LC11-Total FA Method

	DESCRIPTION OF PROCEDURES USED FOR TOTAL (CONJUGATED AND FREE) FATTY ACIDS (if evaluated):						
Sample preparation	Volume of sample extracted (mL):	0.20	A	B	C	D	
	Weights of samples extracted (g):	Unknown Serum 2017 Samples	0.20	0.20	0.20		
		SRM 1950, control	0.20				
Sample processing	Hydrolysis method	Liu et al 2010					
	Extraction method	Solvent extraction					
	Extraction solvent	Hexane:2-proponol (3:2, v/v)					
	Extraction time						
	Extraction - other details						
	Sample extract cleanup method	Phase separartion and SPE catridge cleanup					
	Derivatization reagent						
Instrumental method	Analytical instrument	Thermo Trace GC-DSQ system					
	Column phase	Rxi-5MS-coated 5%diphenyl/95%dimethyl polysiloxane capillary column					
	Column length, m	1/30/1900					
	Column i.d., mm	1/0/1900					
	Column film thickness, µm	0.25					
	Injection method (split, splitless, etc.)	splitless					
Quantitation	Quant Method: ES = external standards (Y/N)	N					
	Number of ES used	NA					
	Quant Method: IS = internal standards (Y/N)	Y					
	Number of IS used	one					
	IS used	Methyl tridecanoate					
	IS added PRIOR to extraction of sample (Y/N)	Yes					
	Calibration model (linear, quad)						
	Calibration range						
Misc.	Additional information, method reference	Liu et al 2010					

Table D-24: Method Descriptions for LC11-Free FA/FAME in Solutions Method

	DESCRIPTION OF PROCEDURES USED FOR FREE FATTY ACID/FATTY ACID METHYL ESTER SOLUTIONS:					
Sample preparation	Volume of sample extracted (mL):		Unknown Free FA Sol. A	Unknown Free FA Sol. B	Unknown Free FA Sol. C	Unknown FAME/FA Sol. D
	Weights of samples extracted (g):	Solutions	0.20	0.20	0.20	1/0/1900
Sample processing	Hydrolysis method (if applies)					
	Extraction method	Solvent extraction				
	Extraction solvent	Hexane:2-proponol (3:2, v/v)				
	Extraction time					
	Extraction - other details					
	Sample extract cleanup method	Phase separartion and SPE catridge cleanup				
	Derivatization reagent	_____				
Instrumental method	Analytical instrument	Thermo Trace GC-DSQ system				
	Column phase	Rxi-5MS-coated 5%diphenyl/95%dimethyl polysiloxane capillary column				
	Column length, m	1/30/1900				
	Column i.d., mm	1/0/1900				
	Column film thickness, µm	0.25				
	Injection method (split, splitless, etc.)	splitless				
Quantitation	Quant Method: ES = external standards (Y/N)	N				
	Number of ES used	NA				
	Quant Method: IS = internal standards (Y/N)	Y				
	Number of IS used	one				
	IS used	Methyl tridecanoate				
	IS added PRIOR to extraction of sample (Y/N)	Yes				
	Calibration model (linear, quad)					
	Calibration range					
Misc.	Additional information, method reference	Liu et al 2010				

Table D-25: Method Descriptions for LC12-Total FA Method

	DESCRIPTION OF PROCEDURES USED FOR TOTAL (CONJUGATED AND FREE) FATTY ACIDS (if evaluated):						
Sample preparation	Volume of sample extracted (mL):	0.10	A	B	C	D	
	Weights of samples extracted (g):	Unknown Serum 2017 Samples					
		SRM 1950, control					
Sample processing	Hydrolysis method	Acidic and alkaline					
	Extraction method	mechanical shaker					
	Extraction solvent	hexane					
	Extraction time	15 minutes					
	Extraction - other details	3x					
	Sample extract cleanup method	Evaporation					
	Derivatization reagent	PFB-Br					
Instrumental method	Analytical instrument	Agilent 7890 GC and 5795 MS					
	Column phase	Select-FAME					
	Column length, m	200 m					
	Column i.d., mm	0.25					
	Column film thickness, µm	0.25					
	Injection method (split, splitless, etc.)	100:1 split					
Quantitation	Quant Method: ES = external standards (Y/N)	N					
	Number of ES used						
	Quant Method: IS = internal standards (Y/N)	Y					
	Number of IS used	18.00					
	IS used	$^{13}C_5$-C16:1n-7t; $^{13}C_5$-C18:1n-9t; $^{13}C_5$-C18:1n-7t; $^{13}C_5$-C18:2n-6t,9t; D_{27}-C14:0; $^{13}C_{16}$-C16:0; $^{13}C_{16}$-C16:1n-7; D_{35}-C18:0; $^{13}C_{18}$-C18:1n-9; $^{13}C_5$-C18:1n-7; $^{13}C_{18}$-C18:2n-6,9; D_{39}-C20:0; D_{14}-C18:3n-3,6,9; D_{43}-C22:0; D_8-C20:4n-6,9,12,15; D_5-C20:5n-3,6,9,12,15; D_{47}-C24:0; D_5-C22:6n-3,6,9,12,15,18					
	IS added PRIOR to extraction of sample (Y/N)	Y					
	Calibration model (linear, quad)	Linear					
	Calibration range	varies by analyte, low end: 0.14 – 7.00 µmol/L, high end: 161 – 8007 µmol/L					
Misc.	Additional information, method reference	Lagerstedt, S.A., D.R. Hinrichs, S.M. Batt, M.J. Magera, P. Rinaldo, and J.P. McConnell. 2001. Quantitative determinaton of plasma c8-c26 total fatty acids for the biochemical diagnosis of nutritional and metabolic disorders. Molecular genetics and matabolism 73: 38-45. Vesper, H.W., S.P. Caudill, H.C. Kuiper, Q. Yang, N. Ahluwalia, D.A. Lacher, and J.L. Pirkle. 2017. Plasma trans-fatty acid concentrations in fasting adults declined from NHANES 1999-2000 to 2009-2010. The American journal of clinical nutrition 105: 1063-1069.					

Table D-26: Method Description for LC12-Free FA/FAME in Solutions.

	DESCRIPTION OF PROCEDURES USED FOR FREE FATTY ACID/FATTY ACID METHYL ESTER SOLUTIONS:					
Sample preparation	Volume of sample extracted (mL):		Unknown Free FA Sol. A	Unknown Free FA Sol. B	Unknown Free FA Sol. C	Unknown FAME/FA Sol. D
	Weights of samples extracted (g):	Solutions				
Sample processing	Hydrolysis method (if applies)					
	Extraction method					
	Extraction solvent					
	Extraction time					
	Extraction - other details					
	Sample extract cleanup method					
	Derivatization reagent					
Instrumental method	Analytical instrument					
	Column phase					
	Column length, m					
	Column i.d., mm					
	Column film thickness, µm					
	Injection method (split, splitless, etc.)					
Quantitation	Quant Method: ES = external standards (Y/N)					
	Number of ES used					
	Quant Method: IS = internal standards (Y/N)					
	Number of IS used					
	IS used					
	IS added PRIOR to extraction of sample (Y/N)					
	Calibration model (linear, quad)					
	Calibration range					
Misc.	Additional information, method reference	Same procedure as for total fatty acids				

Table D-27: Method Descriptions for LC13-Free Glycerol Method

	DESCRIPTION OF PROCEDURES USED FOR FREE GLYCEROL (if evaluated):						
Sample preparation	Volume of sample extracted (mL):	100mL	A	B	C	D	
	Weights of samples extracted (g):	Unknown Serum 2017 Samples	0.00182	0.00164	0.00203		
		SRM 1950, control	0.05376				
Sample processing	Hydrolysis method (if applies)						
	Extraction method	80v/v% Ethanol precipitation					
	Extraction solvent	Ethanol					
	Extraction time	30 minutes					
	Extraction - other details	200uL of samples were admixed 1600uL ethanol contained with 200uL of the internal standard solution using an automatic pipettor. The mixtures were vortexed and stand for 30min at room temperature followed by centrifugation (3000g x 15min).					
	Sample extract cleanup method	The supernatant of ethanol precipitations were derivatized with a 3:1 mixture of pyridine and acetic anhydride at 70 °C for 60min at the heating block. And the derivatized samples were treated by water, hydrogen chloride (6N) and Sodium bicarbonate splution (8%) to remove excess pyridine, acetic anhydride and by-product acetic acid.					
	Derivatization reagent	3:1 mixture of pyridine and acetic anhydride					
Instrumental method	Analytical instrument	the quadrupole mass spectrometer JMS-Q1050GC (JEOL Ltd.)					
	Column phase	DB17-ms (50% phenyl) - methyl-polysiloxane					
	Column length, m	30m					
	Column i.d., mm	0.25					
	Column film thickness, µm	0.25					
	Injection method (split, splitless, etc.)	splitless					
Quantitation	Quant Method: ES = external standards (Y/N)	N					
	Number of ES used						
	Quant Method: IS = internal standards (Y/N)	Y					
	Number of IS used	1.00					
	IS used	13C2-glycerol					
	IS added PRIOR to extraction of sample (Y/N)	Y					
	Calibration model (linear, quad)	linear					
	Calibration range						
Misc.	Additional information, method reference	Clinical chemistry 58(4) 768-776 2012 Reference Measurement Procedure for Total Glycerides by Isotope Dilution GC-MS					

Table D-28: Method Descriptions for LC13-Total Glycerides Method

		DESCRIPTION OF PROCEDURES USED FOR TOTAL GLYCERIDES (if evaluated):					
Sample preparation		Volume of sample extracted (mL):	100mL	A	B	C	D
		Weights of samples extracted (g):	Unknown Serum 2017 Samples	1.541	1.547	1.545	
			SRM 1950, control	1.097			
Sample processing		Hydrolysis method (if applies)	0.3mol/L KOH in ethanol , 60 degree C , 60minutes				
		Extraction method					
		Extraction solvent					
		Extraction time					
		Extraction - other details	Two hundred microliter of samples that have been contained with 200uL of 1,3-13C2-glycerol internel standard solution were hydrolyzed in 0.30 mol/L alcoholic KOH at 60 °C for 1 h followed by the drying under nitrogen using the Turbovap LV evaporator.				
		Sample extract cleanup method	The dried residues were derivatized with a 3:1 mixture of pyridine and acetic anhydride at 70 °C for 60min at the heating block. And the derivatized samples were treated by water, hydrogen chloride (6N) and Sodium bicarbonate splution (8%) to remove excess pyridine, acetic anhydride and by-product acetic acid.				
		Derivatization reagent	3:1 mixture of pyridine and acetic anhydride				
Instrumental method		Analytical instrument	the quadrupole mass spectrometer JMS-Q1050GC (JEOL Ltd.)				
		Column phase	DB17-ms (50% phenyl) - methyl-polysiloxane				
		Column length, m	30m				
		Column i.d., mm	0.25				
		Column film thickness, µm	0.25				
		Injection method (split, splitless, etc.)	splitless				
Quantitation		Quant Method: ES = external standards (Y/N)	N				
		Number of ES used					
		Quant Method: IS = internal standards (Y/N)	Y				
		Number of IS used	1.00				
		IS used	13C2-glycerol				
		IS added PRIOR to extraction of sample (Y/N)	Y				
		Calibration model (linear, quad)	linear				
		Calibration range					
Misc.		Additional information, method reference	Reference : Clinical chemistry 58(4) 768-776 2012 Reference Measurement Procedure for Total Glycerides by Isotope Dilution GC-MS				

Table D-29: Method Descriptions for LC14-Total FA Method

	DESCRIPTION OF PROCEDURES USED FOR TOTAL (CONJUGATED AND FREE) FATTY ACIDS (if evaluated):						
Sample preparation	Volume of sample extracted (mL):		A	B	C	D	
	Weights of samples extracted (g):	Unknown Serum 2017 Samples	0.10	0.10	0.10		
		SRM 1950, control	0.10				
Sample processing	Hydrolysis method	Acid/Base					
	Extraction method	Liquid Phase Extraction usuing automated liquid handler (Hamilton)					
	Extraction solvent	Hexane					
	Extraction time	~1.5 hrs					
	Extraction - other details						
	Sample extract cleanup method						
	Derivatization reagent	Pentafluorobenzyl Bromide (PFBBr)					
Instrumental method	Analytical instrument	Agilent 7890A GC oven + 5975C MS					
	Column phase	TG-Polar					
	Column length, m	2/29/1900					
	Column i.d., mm	0.25					
	Column film thickness, µm	0.20					
	Injection method (split, splitless, etc.)	split					
Quantitation	Quant Method: ES = external standards (Y/N)	N					
	Number of ES used						
	Quant Method: IS = internal standards (Y/N)	Y					
	Number of IS used	18.00					
	IS used						
	IS added PRIOR to extraction of sample (Y/N)	Y					
	Calibration model (linear, quad)	analyte dependant					
	Calibration range						
Misc.	Additional information, method reference						

Table D-30: Method Descriptions for LC14-Free FA/FAME in Solutions Method

	DESCRIPTION OF PROCEDURES USED FOR FREE FATTY ACID/FATTY ACID METHYL ESTER SOLUTIONS:						
Sample preparation	Volume of sample extracted (mL):		Unknown Free FA Sol. A	Unknown Free FA Sol. B	Unknown Free FA Sol. C	Unknown FAME/FA Sol. D	
	Weights of samples extracted (g):	Solutions	0.08045	0.07918	0.07904	0.07968	
Sample processing	Hydrolysis method (if applies)	Acid/Base					
	Extraction method	Liquid Phase Extraction usuing automated liquid handler (Hamilton)					
	Extraction solvent	Hexane					
	Extraction time	~1.5 hrs					
	Extraction - other details						
	Sample extract cleanup method						
	Derivatization reagent	Pentafluorobenzyl Bromide (PFBBr)					
Instrumental method	Analytical instrument	Agilent 7890A GC oven + 5975C MS					
	Column phase	TG-Polar					
	Column length, m	2/29/1900					
	Column i.d., mm	0.25					
	Column film thickness, µm	0.20					
	Injection method (split, splitless, etc.)	split					
Quantitation	Quant Method: ES = external standards (Y/N)	N					
	Number of ES used						
	Quant Method: IS = internal standards (Y/N)	Y					
	Number of IS used	18.00					
	IS used						
	IS added PRIOR to extraction of sample (Y/N)	Y					
	Calibration model (linear, quad)	analyte dependant					
	Calibration range						
Misc.	Additional information, method reference						