National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2378

Fatty Acids in Frozen Human Serum

This Standard Reference Material (SRM) is intended primarily for validation of methods for determining fatty acids in human serum and similar materials. A unit of SRM 2378 consists of three vials, each containing approximately 1.0 mL of frozen serum with different concentrations of fatty acids (Serum 1, Serum 2, and Serum 3).

SRM 2378 was produced in response to a need expressed by the Centers for Disease Control and Prevention (CDC) and in collaboration with the National Institutes of Health, Office of Dietary Supplements (NIH-ODS). Analyses for value assignment were performed by NIST and the CDC.

Certified Mass Fraction Values: The certified mass fraction and concentration values of fatty acids are provided in Table 1. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [1].

Reference Mass Fraction Values: Reference mass fraction and concentration values are listed for additional fatty acids in Table 2. A NIST reference value is a noncertified value that is the best estimate of the true value based on available data; however, the value does not meet the NIST criteria for certification and is provided with associated uncertainties that may reflect only measurement reproducibility, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods [1].

Expiration of Certification: The certification of **SRM 2378** is valid, within the measurement uncertainty specified, until **30 September 2025**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see "Instructions for Storage and Use"). This certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Overall direction and coordination of the technical measurements leading to the certification of this SRM were performed by K.E. Sharpless, M.M. Schantz, B.A. Benner, Jr., L.C. Sander, and S.A. Wise of the NIST Chemical Sciences Division.

Acquisition and preparation of the SRM were coordinated by K.E. Sharpless, and M.M. Schantz.

Analytical measurements at NIST were performed by M.M. Schantz, B.A. Benner, Jr., and L.T. Sniegoski of the NIST Chemical Sciences Division. Analytical measurements at the CDC were provided by C.M. Pfeiffer, C.D. Powers, and R.L. Schleicher.

Support for the development of SRM 2378 Fatty Acids in Human Serum was provided in part by NIH-ODS. Technical consultation was provided by J.M. Betz of NIH-ODS.

Statistical analysis was provided by J.H. Yen of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

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Certificate Revision History on Last Page

Steven J. Choquette, Acting Director
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NOTICE AND WARNING TO USERS

SRM 2378 IS INTENDED FOR RESEARCH USE. THIS IS A HUMAN SOURCE MATERIAL. HANDLE PRODUCT AS A BIOHAZARDOUS MATERIAL CAPABLE OF TRANSMITTING INFECTIOUS DISEASE. The supplier has reported that each donor unit of serum used in the preparation of this product was tested by Food and Drug Administration licensed tests and found to be negative for human immunodeficiency virus (HIV), HIV-1 antigen, hepatitis B surface antigen, and hepatitis C. However, no known test method can offer complete assurance that hepatitis B virus, hepatitis C virus, HIV, or other infectious agents are absent from this material. Accordingly, this human blood-based product should be handled at the Biosafety Level 2 or higher as recommended for any POTENTIALLY INFECTIOUS HUMAN SERUM OR BLOOD SPECIMEN in the CDC/NIH Manual [2].

INSTRUCTIONS FOR STORAGE AND USE

Storage: The SRM should be stored at –80 °C in the original unopened vials. The certification does not apply to contents of previously opened vials as the stabilities of the analytes have not been investigated.

Use: SRM 2378 is provided as frozen serum that should thaw at room temperature in approximately 30 min under subdued light prior to use. After the material is thawed, it should be used immediately. The contents of the vial should be gently mixed prior to removal of a test portion for analysis. Precautions should be taken to avoid exposure to strong UV light and direct sunlight.

SOURCE, PREPARATION, AND ANALYSIS[(1)]

Source and Preparation: Donor serum for SRM 2378 was collected from three groups of individuals: Serum 1, three healthy donors who took 1000 mg/day of fish oil supplements for a minimum of one month prior to collection; Serum 2, three healthy donors who took 1000 mg/day of flaxseed oil supplements for a minimum of one month prior to collection; and Serum 3, three healthy donors who did not take either fish or flaxseed oil supplements for one month prior to collection.

Homogeneity Assessment: The homogeneity of the material was assessed at NIST using the *NIST-1* sulfuric acid in methanol derivatization method for the certified and reference values and test portion sizes described below; analysis of variance did not show statistically significant heterogeneity. The reported analytes have been treated as though they are homogeneously distributed in the material; the homogeneity of the other analytes present in the material and not reported by NIST and/or CDC was not assessed.

Value Assignment: Means of data sets from individual methods were combined to provide assigned values. The NIST measured serum densities are 1.02259 g/mL ± 0.00006 g/mL, 1.02320 g/mL ± 0.00011 g/mL, and 1.02212 g/mL ± 0.00001 g/mL, for SRM 2378 Sera 1, 2, and 3, respectively. The uncertainties for the serum densities were incorporated in values that are reported in Tables 1 and 2 relative to units of volume.

Analytical Approach for Determination of Fatty Acids

Determination of Fatty Acids (NIST-1): For the homogeneity measurements performed at NIST, an internal standard solution containing palmitic-d_{31} acid and heneicosanoic acid was used. The samples (two aliquots, 0.5 g each, from each of 10 vials for each of the three sera) were saponified in methanolic KOH and esterified using sulfuric acid in methanol [3]. The samples were analyzed by gas chromatography (GC) with flame ionization detection (FID) using a 0.25 mm × 100 m biscyanopropyl polysiloxane fused silica capillary column.

Determination of Fatty Acids (NIST-2 and CDC): NIST-2 and the CDC methods determined total fatty acids using isotope dilution GC/mass spectrometry (ID-GC-MS) based on Langerstedt's method [4] using four aliquots of 0.1 g serum for the NIST-2 method (actual masses determined) and three 0.10 mL aliquots for the CDC method. This procedure hydrolyzes fatty acids from cholesteryl esters, triglycerides, and phospholipids using sequential addition of acetonitrile:hydrochloric acid and methanol:sodium hydroxide in the presence of heat. The hydrolysis procedure for the NIST-2 measurements used microwave-assisted digestions of the sera samples for 45 min at 100 °C (± 5 °C) using acetonitrile:hydrochloric acid. Total fatty acids were extracted in hexane, concentrated, derivatized using pentafluorobenzyl bromide (PFB), and reconstituted in hexane. Fatty acids were quantified using four (NIST-2) and

[(1)] Certain commercial equipment, instruments, or materials are identified in this certificate in order to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

ten (CDC) isotopically labeled internal standards after separation on a 30 m × 0.25 mm × 0.25 µm, 5 % (mole fraction) phenylmethyl-substituted polysiloxane column (NIST-2) and 60 m x 0.25 mm x 0.25 µm, 50 % cyanopropyl + 50 % phenylpolysiloxane column (CDC). Fragmentation of fatty acid-PFB esters by negative chemical ionization (electron capture ionization) resulted in a reproducible loss of the PFB moiety giving a stable carboxylate anion $(M-1)^-$.

Interlaboratory Analytical Comparison Study of Total Fatty Acid Concentrations in Human Serum: SRM 2378 was distributed as study samples to 12 laboratories for an interlaboratory comparison in May 2012 [8]. The laboratories were provided with four vials of each material and were requested to do triplicate measurements for each material (three sera of SRM 2378) using their laboratory's and/or program's analytical protocols for the fatty acids currently determined in their laboratory. The results from this study were used to confirm the values determined during certification measurements by the methods described above.

Certified Values for Fatty Acids: Certified values for fatty acids are means of the method means from analyses at NIST using ID-GC-MS and/or GC-FID and from CDC using ID-GC-MS. The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand, the fatty acid concentration. The expanded uncertainty is calculated as $U = ku_c$, where u_c incorporates the observed difference between the results from the methods and their respective uncertainties, consistently with the ISO/JCGM Guide and with its Supplement 1, and k is a coverage factor, where $k = 2.0$, corresponding to approximately 95 % confidence [5–7]. See the footnotes for the specific analytical methods used in determining the certified values. The measurands are the lipids listed in Table 1, and metrological traceability is to the SI derived units for mass fraction (expressed as micrograms per gram) and amount-of-substance concentration (expressed as micromoles per liter).

Table 1. Certified Values for Fatty Acids in SRM 2378

		Serum 1		Serum 2		Serum 3	
Lipid Name	Chemical Name (Common Name)	Mass Fraction (µg/g)	Molarity (µmol/L)	Mass Fraction (µg/g)	Molarity (µmol/L)	Mass Fraction (µg/g)	Molarity (µmol/L)
C14:0	Tetradecanoic acid[(a)] (Myristic acid)	44.6 ± 1.5	200 ± 7	33.8 ± 1.2	151 ± 5	34.6 ± 0.8	155 ± 4
C16:0	Hexadecanoic acid[(a)] (Palmitic acid)	833 ± 87	3320 ± 350	715 ± 111	2850 ± 440	642 ± 114	2560 ± 460
C16:1 n-7	(Z)-9-Hexadecenoic Acid[(b)] (Palmitoleic acid)	53.4 ± 3.8	214 ± 15	69.1 ± 6.9	278 ± 28	45.7 ± 3.2	184 ± 13
C18:0	Octadecanoic acid[(a)] (Stearic acid)	221 ± 25	795 ± 90	231 ± 14	830 ± 50	194 ± 21	696 ± 75
C18:1 n-9	(Z)-9-Octadecenoic Acid[(a)] (Oleic acid)	604 ± 66	2190 ± 240	738 ± 52	2670 ± 189	569 ± 66	2060 ± 240
C18:2 n-6	(Z,Z)-9,12-Octadecadienoic acid[(b)] (Linoleic acid)	1030 ± 180	3740 ± 640	1220 ± 10	4460 ± 45	913 ± 6	3330 ± 20
C18:3 n-3	(Z,Z,Z)-9,12,15-Octadecatrienoic acid[(b)] (alpha-Linolenic acid)	32.5 ± 4.1	119 ± 15	31.5 ± 1.3	116 ± 5	17.0 ± 0.1	62.4 ± 0.5
C18:3 n-6	(Z,Z,Z)-6,9,12-Octadecatrienoic acid[(a)] (gamma-Linolenic acid)	12.3 ± 1.6	45.1 ± 6.0	21.0 ± 0.4	77.1 ± 1.4	14.6 ± 1.0	53.6 ± 3.6
C20:4 n-6	(Z,Z,Z,Z)-5,8,11,14-Eicosatetraenoic acid[(a)] (Arachidonic acid)	196 ± 20	659 ± 69	235 ± 26	790 ± 86	228 ± 14	765 ± 46
C20:5 n-3	(Z,Z,Z,Z,Z)-5,8,11,14,17-Eicosapentaenoic acid[(a)] (EPA)	84 ± 11	284 ± 37	20.7 ± 0.8	70.1 ± 2.6	18.9 ± 2.2	63.8 ± 7.6
C22:5 n-3	(Z,Z,Z,Z,Z)-7,10,13,16,19-Docosapentaenoic acid[(a)] (DPA)	22.4 ± 1.2	69.4 ± 3.6	17.0 ± 0.8	52.6 ± 2.4	11.4 ± 0.6	35.1 ± 1.9
C22:6 n-3	(Z,Z,Z,Z,Z,Z)-4,7,10,13,16,19-Docosahexaenoic acid[(a)] (DHA)	104 ± 5	323 ± 16	55.4 ± 2.3	173 ± 7	54.9 ± 2.4	171 ± 8

[(a)] NIST-1, NIST-2, and CDC method ($k = 2.0$)
[(b)] NIST-1 and CDC method ($k = 2.0$)

Reference Values for Fatty Acids: Reference values for fatty acids are provided below with method designations described in footnotes. The values are calculated as the means of the values provided by the methods used. The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as $U = ku_c$, where u_c is the combined uncertainty, consistent with the ISO/JCGM Guide, and k is a coverage factor corresponding to approximately 95 % confidence [5–7]. See the footnotes for the coverage factors used in the calculations. The measurands are the lipids listed in Table 2 as determined by the indicated methods, and metrological traceability is to the SI derived units for mass fraction (expressed as micrograms per gram) and amount-of-substance concentration (expressed as micromoles per liter).

Table 2. Reference Values for Fatty Acids in SRM 2378

		Serum 1		Serum 2		Serum 3	
		Mass Fraction (µg/g)	Molarity (µmol/L)	Mass Fraction (µg/g)	Molarity (µmol/L)	Mass Fraction (µg/g)	Molarity (µmol/L)
Lipid Name	Chemical Name (Common Name)						
C10:0	Decanoic acid (Capric acid)[a]	3.56 ± 0.04	21.1 ± 0.2	0.3 ± 0.1	1.9 ± 0.8	0.89 ± 0.15	5.3 ± 0.9
C14:1 n-5	(*Z*)-Tetradec-9-enoic acid (Myristoleic acid)[b]	3.4 ± 1.5	16 ± 7	2.8 ± 1.5	13 ± 7	3.2 ± 1.7	14 ± 8
C15:0	Pentadecanoic acid[a]	5.14 ± 0.16	21.7 ± 0.7	6.76 ± 0.16	28.5 ± 0.7	4.92 ± 0.12	20.7 ± 0.5
C17:0	Heptadecanoic acid (Margaric acid)[a]	7.11 ± 0.47	26.9 ± 1.8	8.84 ± 0.21	33.4 ± 0.8	7.02 ± 0.37	26.5 ± 1.4
C18:1 n-7	(*Z*)-11-Octadecenoic acid (cis-Vaccenic acid)[b]	41.3 ± 7.4	149 ± 27	35.6 ± 6.0	129 ± 22	32.0 ± 3.3	116 ± 12
C18:4 n-3	(Z,Z,Z,Z)-6,9,12,15-octadecatetraenoic acid (Stearidonic acid)[a]	2.20 ± 0.31	8.1 ± 1.1	1.11 ± 0.15	4.11 ± 0.54	0.77 ± 0.05	2.83 ± 0.17
C20:0	Eicosanoic acid (Arachidic acid)[c]	7.6 ± 1.1	25.0 ± 3.6	8.7 ± 1.5	28.4 ± 4.8	7.9 ± 2.7	26.0 ± 9.0
C20:1 n-9	(Z)-11-Eicosenoic acid (Gondoic acid)[b]	6.0 ± 1.0	19.7 ± 3.3	5.7 ± 1.0	18.9 ± 3.4	5.88 ± 0.43	19.4 ± 1.4
C20:3 n-9	(Z,Z,Z)-5,8,11-Eicosatrienoic acid[a]	1.40 ± 0.09	4.67 ± 0.29	2.13 ± 0.07	7.10 ± 0.23	2.26 ± 0.03	7.54 ± 0.10
C22:0	Docosanoic acid (Behenic acid)[b]	18.8 ± 4.3	57 ± 13	29 ± 9	86 ± 27	19.2 ± 4.5	58 ± 14
C22:1 n-9	(Z)-13-Docosenoic acid (Erucic acid)[b]	1.7 ± 1.0	5 ± 3	1.7 ± 0.9	5 ± 3	1.9 ± 1.3	6 ± 4
C22:2 n-6	(Z,Z)-13,16-Docosadienoic acid[a]	0.31 ± 0.02	0.93 ± 0.06	0.36 ± 0.03	1.09 ± 0.09	0.303 ± 0.003	0.92 ± 0.01
C22:5 n-6	(Z,Z,Z,Z,Z)-4,7,10,13,16-Docosapentaenoic acid[a]	2.71 ± 0.21	8.37 ± 0.64	5.20 ± 0.41	16.1 ± 1.3	5.16 ± 0.42	16.0 ± 1.3
C23:0	Tricosanoic acid[a]	8.19 ± 0.19	23.6 ± 0.5	13.1 ± 0.5	37.9 ± 1.5	7.75 ± 0.07	22.3 ± 0.2
C24:0	Tetracosanoic acid (Lignoceric acid)[c]	19 ± 5	54 ± 13	25 ± 9	70 ± 25	18 ± 6	49 ± 16
C24:1 n-9	(Z)-15-Tetracosenoic acid (Nervonic acid)[c]	32 ± 9	89 ± 26	30 ± 17	83 ± 49	22 ± 9	61 ± 25
C26:0	Hexacosanoic acid (Cerotic acid)[a]	0.35 ± 0.05	0.89 ± 0.12	0.25 ± 0.04	0.63 ± 0.11	0.34 ± 0.09	0.86 ± 0.2
	(*7R,11R*)-3,7,11,15-Tetramethyl-hexadecanoic acid (phytanic acid)[d]	0.55 ± 0.02	1.80 ± 0.06	0.33 ± 0.02	1.07 ± 0.06	0.48 ± 0.06	1.56 ± 0.18

[a] CDC method (k = 4.30)
[b] NIST-1 and CDC method (k = 2.0)
[c] NIST-1, NIST-2, and CDC method (k = 2.0)
[d] NIST-2 method (k = 3.18)

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Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; fax (301) 948-3730; email srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.