National Institute of Standards and Technology
U.S. Department of Commerce

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Standard Reference Material® 3951
Fatty Acid Species in Frozen Human Serum
CERTIFICATE OF ANALYSIS

Purpose: The certified values delivered by this Standard Reference Material (SRM) are intended for validating methods for determining free and total fatty acids in human serum and similar materials.

Description: A unit of SRM 3951 consists of three vials of frozen serum with each vial containing approximately 1.0 mL of serum.

Certified Values: Certified values of selected fatty acids inherently in their free state in SRM 3951 are provided in Table 1. A NIST certified value is a value for which NIST has the highest confidence in that all known or suspected sources of bias have been evaluated [1]. These values were assigned based upon measurements made at NIST using gas chromatographic methods in combination with results from an interlaboratory study. These values are traceable to derived International System of Units (SI) for mass fraction and amount-of-substance concentration [2].

Table 1. Certified Values for Free Fatty Acids in SRM 3951[(a)]

Lipid Name	Chemical Name (Common Name)	M (g/mol)	Mass Fraction (µg/g)	Concentration (µmol/L)[(b)]
C14:0	Tetradecanoic acid (Myristic acid)	228.4	1.91 ± 0.28	8.6 ± 1.2
C16:0	Hexadecanoic acid (Palmitic acid)	256.4	48.1 ± 2.9	192 ± 17
C16:1n7	(Z)-9-Hexadecenoic acid (Palmitoleic acid)	254.4	3.56 ± 0.60	14.3 ± 2.4
C18:0	Octadecanoic acid (Stearic acid)	284.5	14.4 ± 3.6	52 ± 13
C18:1n9	(Z)-9-Octadecenoic acid (Oleic acid)	282.5	36.1 ± 0.8	131 ± 3
C18:2n6	(Z,Z)-9,12-Octadecadienoic acid (Linoleic acid)	280.4	32.5 ± 3.6	117 ± 14

[(a)] Certified values are expressed as $x \pm U(x)$, where x is the value and $U(x)$ is the expanded uncertainty of the value. The true value of the measurand is believed to lie within the interval $x \pm U(x)$ with 95 % confidence. A certified value can be regarded as a normally distributed random variable with mean x and standard deviation $U(x)/2$. For guidance in propagating this uncertainty, see reference 3.

[(b)] The density of SRM 3951, used to convert from mass fraction (µg/g) to µmol/L, is reported in Table 2.

Non-Certified Values: Non-certified values are provided in Appendix A.

Period of Validity: The certification of **SRM 3951** is valid, within the measurement uncertainty specified, until **30 September 2025**, provided the SRM is handled and stored in accordance with instructions given in this certificate. The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of Certified Values: NIST will monitor this SRM to the end of the period of validity. 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.

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Certified Serum Density Value: The certified serum density is listed in Table 2. This value is metrologically traceable to the SI units of mass and length.

Table 2. Certified Serum Density of SRM 3951 at 22.6 °C[(a)]

Measurand	Density (g/mL)
Serum Density at 22.6 °C ± 0.2 °C	1.0214 ± 0.0004

[(a)] Certified values are expressed as $x \pm U(x)$, where x is the value and $U(x)$ is the expanded uncertainty of the value. The true value of the measurand is believed to lie within the interval $x \pm U(x)$ with 95 % confidence. A certified value can be regarded as a normally distributed random variable with mean x and standard deviation $U(x)/2$. For guidance in propagating this uncertainty, see reference 3.

Safety: SRM 3951 IS INTENDED FOR LABORATORY RESEARCH USE ONLY. 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 an FDA-approved method and found non-reactive for hepatitis B surface antigen (HbsAg), human immunodeficiency virus (HIV) 1 and 2 antibodies, and hepatitis C virus (HCV). However, no known test method can offer complete assurance that hepatitis B virus, HCV, HIV, or other infectious agents are absent from this material. As such, this human blood-based product should be handled at the Biosafety Level 2 or higher as recommended by the Centers for Disease Control and Prevention/National Institutes of Health manual Biosafety in Microbiological and Biomedical Laboratories (5th edition) [4] for human-derived blood products where the presence of an infectious agent may be unknown.

This SRM was developed after an appropriate human subjects research determination by NIST.

Storage: SRM 3951 should be stored at or below −70 °C in the original unopened vials. The certification does not apply to contents of previously opened vials.

Use: SRM 3951 is provided as frozen serum that should be allowed to thaw at room temperature for at least 30 min. 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: Full details on the sourcing, production, analysis, and statistical evaluation of SRM 3951 are provided in reference 5.

Procurement of human serum for SRM 3951 and the Fatty Acid Quality Assurance Program (FAQAP) was supported by The National Institutes of Health Office of Dietary Supplements (NIH-ODS).

In 2017, SRM 3951 was distributed as an unknown material to fourteen laboratories through the Fatty Acid Quality Assurance Program, from which selected results were used for the determination of certified and non-certified values for free and total fatty acids in SRM 3951.

Relationship Between Mass Fraction and Amount-of-Substance Values: Values listed in units of mass fraction (y, expressed in micrograms per gram, µg/g) are related to those listed in amount-of-substance concentration (z, expressed in micromole per liter, µmol/L) through the measured serum density, D, and calculated molar masses, M, of the analyte. The expanded uncertainty associated with M, $U(M)$, is conservatively estimated as 0.12 g/mol. The relationship equations are:

$$z\ \mu\text{mol/L} = (y\ \mu\text{g/g})(1000\ D\ \text{g/mL})/(M\ \text{g/mol});\ \ U(z) = z[(U(y)/y)^2 + (U(D)/D)^2 + (U(M)/M)^2]^{0.5}$$

$$y\ \mu\text{g/g} = (z\ \mu\text{mol/L})(M\ \text{g/mol})/(1000\ D\ \text{g/mL});\ \ U(y) = y[(U(z)/z)^2 + (U(D)/D)^2 + (U(M)/M)^2]^{0.5}$$

REFERENCES

[1] Beauchamp, C.R.; Camara, J.E.; Carney, J.; Choquette, S.J.; Cole, K.D.; DeRose, P.C.; Duewer, D.L.; Epstein, M.S.; Kline, M.C.; Lippa, K.A.; Lucon, E.; Molloy, J.; Nelson, M.A.; Phinney, K.W.; Polakoski, M.; Possolo, A.; Sander, L.C.; Schiel, J.E.; Sharpless, K.E.; Toman, B.; Winchester, M.R.; Windover, D.; *Metrological Tools for the Reference Materials and Reference Instruments of the NIST Material Measurement Laboratory*; NIST Special Publication (NIST SP) 260-136, 2021 edition; U.S. Government Printing Office: Washington, DC (2021); available at https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-136-2021.pdf (accessed Nov 2021).

[2] Thompson, A.; Taylor, B.N.; *Guide for the Use of the International System of Units (SI)*; NIST Special Publication 811; U.S. Government Printing Office: Washington, DC (2008); available at https://www.nist.gov/pml/special-publication-811 (accessed Nov 2021).

[3] Possolo, A.M.; *Evaluating, Expressing, and Propagating Measurement Uncertainty for NIST Reference Materials*; NIST Special Publication (NIST SP) 260-202 (2020); U.S. Government Printing Office: Washington, DC (2020); available at https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-202.pdf (accessed Nov 2021).

[4] CDC/NIH: *Biosafety in Microbiological and Biomedical Laboratories*, 5th ed.; HHS publication No. (CDC) 21-1112; Chosewood, L.C.; Wilson, D.E., Eds.; U.S. Government Printing Office: Washington, DC (2009); available at https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2009-P.PDF (accessed Nov 2021).

[5] Benner, B.A., Jr.; Murray, J.A.; *NIST Fatty Acid Quality Assurance Program 2017 Final Report*; National Institute of Standards and Technology Interagency or Internal Report (NISTIR) 8273; U.S. Government Printing Office: Washington, DC (2019); available at https://nvlpubs.nist.gov/nistpubs/ir/2019/NIST.IR.8273.pdf (accessed Nov 2021).

If you use this SRM in published work, please reference:

Benner BA Jr, Murray JA (2019) NIST Fatty Acid Quality Assurance Program 2017 Final Report. (National Institute of Standards and Technology, Gaithersburg, MD), National Institute of Standards and Technology Interagency or Internal Report (NISTIR) 8273. https://doi.org/10.6028/NIST.IR.8273

Certain commercial equipment, instruments, or materials may be identified in this Certificate of Analysis 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.

Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the Office of Reference Materials 100 Bureau Drive, Stop 2300, Gaithersburg, Maryland 20899-2300; telephone (301) 975-2200; e-mail srminfo@nist.gov; or via the Internet at https://www.nist.gov/srm.

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APPENDIX A

Non-Certified Values: The non-certified values for free fatty acids are listed in Table A1 and those for total fatty acids are listed in Table A2. Non-certified values are best estimates based on currently available information. However, they do not meet NIST's criteria for certification [1]. Non-certified values cannot be used to establish metrological traceability to the International System of Units or other higher-order reference system.

Table A1. Non-Certified Values for Free Fatty Acids in SRM 3951[(a)]

Lipid Name	Chemical Name (Common Name)	*M* (g/mol)	Mass Fraction (µg/g)	Concentration (µmol/L)[(b)]
C18:3n3	(Z,Z,Z)-9,12,15-Octadecatrienoic acid (α-Linolenic acid)	278.4	0.87 ± 0.29	3.2 ± 1.1
C18:3n6	(Z,Z,Z)-6,9,12-Octadecatrienoic acid (γ-Linolenic acid)	278.4	0.23 ± 0.08	0.84 ± 0.28
C20:1n9	(Z)-11-Eicosenoic acid (Gondoic acid)	310.5	0.29 ± 0.14	0.97 ± 0.46
C20:2n6	11,14-Eicosadienoic acid	308.5	0.25 ± 0.05	0.82 ± 0.16
C22:6n3	(Z,Z,Z,Z,Z,Z)-4,7,10,13,16,19-Docosa-hexaenoic acid (DHA)	328.5	1.00 ± 0.38	3.1 ± 1.2

[(a)] These consensus values are expressed as $x \pm 2u(x)$, where x is the value and $u(x)$ is the standard uncertainty of x. While the best estimate of the mass fraction for these measurands lies within the interval $x \pm 2u(x)$, this interval may not include the true value. For guidance in propagating this uncertainty, see reference 3.
[(b)] The density of SRM 3951, used to convert from mass fraction (µg/g) to µmol/L, is reported in Table 2.

Table A2. Non-Certified Values for Total Fatty Acids in SRM 3951[(a)]

Lipid Name	Chemical Name (Common Name)	*M* (g/mol)	Mass Fraction (µg/g)	Concentration (µmol/L)[(b)]
C14:0	Tetradecanoic acid (Myristic acid)	228.4	32.1 ± 0.6	144 ± 3
C14:1n5	(*Z*)-Tetradec-9-enoic acid (Myristoleic acid)	226.4	2.01 ± 0.50	9.1 ± 2.2
C15:0	Pentadecanoic acid	242.4	2.44 ± 0.47	10.3 ± 2.0
C16:0	Hexadecanoic acid (Palmitic acid)	256.4	717 ± 16	2860 ± 62
C16:1n7	(Z)-9-Hexadecenoic acid (Palmitoleic acid)	254.4	54.9 ± 6.2	221 ± 25
C17:0	Heptadecanoic acid (Margaric acid)	270.5	9.1 ± 3.0	34 ± 11
C18:0	Octadecanoic acid (Stearic acid)	284.5	248 ± 13	891 ± 46
C18:1n7	(Z)-11-Octadecenoic acid (cis-Vaccenic acid)	282.5	41.9 ± 6.7	151 ± 24
C18:1n9	(Z)-9-Octadecenoic acid (Oleic acid)	282.5	585 ± 54	2117 ± 197
C18:2n6	(Z,Z)-9,12-Octadecadienoic acid (Linoleic acid)	280.4	968 ± 179	3524 ± 653
C18:3n3	(Z,Z,Z)-9,12,15-Octadecatrienoic acid (α-Linolenic acid)	278.4	18.1 ± 1.5	66.4 ± 5.6
C20:0	Eicosanoic acid (Arachidic acid)	312.5	7.83 ± 0.56	25.6 ± 1.8
C20:1n9	(Z)-11-Eicosenoic acid (Gondoic acid)	310.5	4.17 ± 0.70	13.7 ± 2.3
C20:2n6	11,14-Eicosadienoic acid	308.5	6.71 ± 0.72	22.2 ± 2.4
C20:3n6	(Z,Z,Z)-8,11,14-Eicosatrienoic acid (dihomo-γ-Linolenic acid)	306.5	50.1 ± 0.5	167 ± 2
C20:4n6	(Z,Z,Z,Z)-5,8,11,14-Eicosatetraenoic acid (Arachidonic acid)	304.5	227 ± 12	762 ± 39
C20:5n3	(Z,Z,Z,Z,Z)-5,8,11,14,17-Eicosapentaenoic acid (EPA)	302.5	13.5 ± 1.0	45.7 ± 3.3
C22:0	Docosanoic acid (Behenic acid)	340.6	23.5 ± 2.9	70 ± 9
C22:4n6	(Z,Z,Z,Z)-7,10,13,16-Docosatetraenoic acid	332.5	7.7 ± 0.5	23.7 ± 1.6
C22:5n3	(*Z,Z,Z,Z,Z*)-7,10,13,16,19-Docosapentaenoic acid (DPA)	330.5	13.5 ± 0.5	41.8 ± 1.6
C22:5n6	(Z,Z,Z,Z,Z)-4,7,10,13,16-Docosapentaenoic acid	330.5	9.2 ± 0.5	28.4 ± 1.6
C22:6n3	(Z,Z,Z,Z,Z)-4,7,10,13,16,19-Docosohexaenoic acid (DHA)	328.5	57.2 ± 6.3	178 ± 20
C24:0	Tetracosanoic acid (Lignoceric acid)	368.6	20.4 ± 0.3	56.6 ± 0.8

[(a)] These consensus values are expressed as $x \pm 2u(x)$, where x is the value and $u(x)$ is the standard uncertainty of x. While the best estimate of the mass fraction for these measurands lies within the interval $x \pm 2u(x)$, this interval may not include the true value. For guidance in propagating this uncertainty, see reference 3.
[(b)] The density of SRM 3951, used to convert from mass fraction (µg/g) to µmol/L, is reported in Table 2.

Maintenance of Non-Certified Values: NIST will support the above non-certified values to the end of the period of validity stated on page 1. If substantive technical changes occur that affect the non-certified values during this period, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

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