NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY
U.S. DEPARTMENT OF COMMERCE

Date of Issue:
12 February 2024

Standard Reference Material® 1546a
Meat Homogenate
CERTIFICATE OF ANALYSIS

Purpose: The certified values delivered by this Standard Reference Material (SRM) are intended for validating methods for determining cholesterol, vitamins, and elements in canned meat products and similar materials and for quality assurance when assigning values to in-house control materials.

Description: A unit of SRM 1546a consists of four cans, each containing approximately 85 g of a mixture of pork and chicken products blended in a commercial process.

Certified Values: NIST certified values are traceable to the International System of Units (SI) derived unit of mass fraction, expressed as milligrams per kilogram or milligram per gram. The values are reported on an as-received basis [1].

Table 1. Certified Values for Cholesterol, Vitamins, and Elements in SRM 1546a

Measurand	Mass Fraction[(a)] (mg/g)
Cholesterol	0.717 ± 0.022

Measurand	Mass Fraction[(a)] (mg/kg)
Niacin (Vitamin B_3)[(b)]	0.401 ± 0.022
Niacinamide (Vitamin B_3)[(b)]	38.18 ± 0.74
Total Vitamin B_3 as Niacinamide[(c)]	41.0 ± 4.8
25-Hydroxyvitamin D_3	0.00090 ± 0.00012

Measurand	Mass Fraction[(a)] (mg/kg)
Copper (Cu)	0.605 ± 0.051
Iron (Fe)	10.17 ± 0.35
Magnesium (Mg)	178.1 ± 4.8
Manganese (Mn)	0.286 ± 0.024
Phosphorus (P)	1651 ± 32
Potassium (K)	2490 ± 210
Selenium (Se)	0.281 ± 0.017
Sodium (Na)	9600 ± 1100
Zinc (Zn)	17.88 ± 0.35

(a) Values are expressed as $x \pm U_{95\%}(x)$, where x is the certified value and $U_{95\%}(x)$ is the expanded uncertainty of the certified value. The true value of the analyte lies within the interval $x \pm U_{95\%}(x)$ with 95 % confidence. To propagate this uncertainty, treat the certified value as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$ [2–3].

(b) This value represents the free (unbound) form of the vitamin.

(c) NIST measured niacinamide and niacin individually, and niacin was mathematically converted to niacinamide by multiplication by the ratio of the relative molecular masses.

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

Period of Validity: The certified values delivered by **SRM 1546a** are valid within the measurement uncertainty specified until **31 January 2034**. The certified values are nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

Maintenance of Certified Values: NIST will monitor this SRM over the period of its validity. If substantive technical changes occur that affect the certification, NIST will issue an amended certificate through the NIST SRM website (https://www.nist.gov/srm) and notify registered users. SRM users can register online from a link available on the NIST SRM website or fill out the user registration form that is supplied with the SRM. Registration will facilitate notification. Before making use of any of the values delivered by this material, users should verify they have the most recent version of this documentation, available through the NIST SRM website (https://www.nist.gov/srm).

Carlos A. Gonzalez, Chief
Chemical Sciences Division

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Office of Reference Materials

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Safety: SRM 1546a IS INTENDED FOR RESEARCH USE; not for human consumption.

Storage: The original unopened cans of SRM 1546a should be stored at room temperature (not to exceed 22 °C) or under refrigeration (2 °C and above) but should not be frozen. The value assignments do not apply to contents of previously opened cans, because the stability of all analytes in previously opened cans has not been investigated.

Use: Before use, the contents of the can should be mixed thoroughly to ensure homogeneity. One technique recommended is to transfer the entire contents of a can to a plastic bag, then manually squeeze the bag to blend the material. Care should be taken to avoid separating fat from the material. Homogeneity of the material has not been evaluated for sample sizes smaller than those used by NIST methods described in Appendix B. Therefore, the certified values may not be valid for test portions smaller than 1 g for determination of cholesterol, 2 g for determination of vitamins, and 3.5 g for determination of elements.

Source and Preparation: SRM 1546a is a mixture of pork, mechanically separated chicken, ham, salt, sucrose, water, and spices and was prepared by the Hormel Foods Corporation (Austin, MN), by a commercial process that included cooking, grinding, blending, and sieving prior to canning under sterile conditions. A small quantity of sodium nitrite was added as a preservative prior to canning.

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Certificate Revision History: **12 February 2024** (Information value for taurine removed; certified values for pantothenic acid and pyridoxamine removed due to observed instability; non-certified values for total vitamin B_5, pyridoxine, and total vitamin B_6 removed due to observed instability; period of validity extended; editorial changes); **30 October 2020** (Certified values for fatty acids downgraded to reference values to properly reflect traceability and moved from Table 1 to Table 6; reference values for fatty acids moved from Table 5 to Table 6; removal of values for margaroleic acid, arachidonic acid, and behenic acid based on NIST's decision to no longer support these values in this matrix; update to the value for vaccenic acid to include only collaborating laboratory data; addition of a reference value for nitrate; included selenium value change in revision history for 21 March 2016 revision; editorial changes); **24 August 2017** (Correction and upgrade of decanoic acid value from reference to certified; rounding corrections to mass fraction values for histidine, valine, total *trans* fat, and elaidic acid; editorial changes); **21 March 2016** (Addition of certified value for 25-hydroxyvitamin D_3; addition of reference values for vitamin D_3, carnitine, and rubidium; updates of certified value for selenium, reference value for chlorine; update of value for choline from an information value to a reference value; conversion of vitamin B_6 values to the non-salt form; addition of method information; editorial changes); **02 May 2014** (Original certificate date).

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, MD 20899-2300; telephone (301) 975-2200; e-mail srminfo@nist.gov; or the Internet at https://www.nist.gov/srm.

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APPENDIX A

Non-Certified Values: Non-certified values are suitable for use in method development, method harmonization, and process control but do not meet the NIST criteria for certification [1] nor provide metrological traceability to the International System of Units (SI) or other higher-order reference system. They are the best estimates of the true values based on available data. The values are provided with an uncertainty that may reflect only measurement reproducibility, may not include all sources of uncertainty, and/or may reflect a lack of sufficient statistical agreement among multiple analytical methods. Information about methods used to determine non-certified values is summarized in Appendix B.

Table A1. Non-Certified Values for Vitamins, Elements, and Nitrate in SRM 1546a

Measurand	Mass Fraction[a] (mg/kg)	Measurand	Mass Fraction[a] (mg/kg)
Thiamine (Vitamin B_1)[b,c]	0.90 ± 0.48	Barium (Ba)	0.077 ± 0.019
Riboflavin (Vitamin B_2)[c]	0.35 ± 0.10	Boron (B)	0.306 ± 0.039
Total Vitamin B_{12} by Microbiological Assay	0.0055 ± 0.0016	Calcium (Ca)	360 ± 130
Cholecalciferol (Vitamin D_3)	0.00256 ± 0.00053	Chlorine (Cl)	15300 ± 2300
Choline	536.4 ± 9.8	Molybdenum (Mo)	0.016 ± 0.002
Carnitine	92.0 ± 1.4	Rubidium (Rb)	2.56 ± 0.11
Nitrate	24.2 ± 4.6	Strontium (Sr)	0.305 ± 0.070

[a] These values are expressed as $x \pm 2u(x)$, where x is a mean value and $u(x)$ is its associated standard uncertainty. The method-specific value of the analyte lies within the interval $x \pm U_{95\%}(x)$ with 95 % confidence.

[b] Reported as thiamine ion (relative molecular mass of 265.36 g/mol), not chloride or chloride hydrochloride.

[c] This value represents the free (unbound) form of the vitamin.

Table A2. Non-Certified Values for Fatty Acids (as Free Fatty Acids) in SRM 1546a

	Common Name	Mass Fraction[a] (g/100 g)
Decanoic Acid (C10:0)[b,c]	Capric Acid	0.0167 ± 0.0017
Dodecanoic Acid (C12:0)[b,c,d]	Lauric Acid	0.0153 ± 0.0011
Tetradecanoic Acid (C14:0)[b,c,d]	Myristic Acid	0.245 ± 0.023
(Z)-9-Tetradecenoic Acid (C14:1 n-5)[b,c,d]	Myristoleic Acid	0.0118 ± 0.0028
Pentadecanoic Acid (C15:0)[c]		0.010 ± 0.002
Hexadecanoic Acid (C16:0)[b,c,d]	Palmitic Acid	4.63 ± 0.53
(Z)-9-Hexadecenoic Acid (C16:1 n-7)[b,c,d]	Palmitoleic Acid	0.618 ± 0.078
Heptadecanoic Acid (C17:0)[c]	Margaric Acid	0.0575 ± 0.0028
Octadecanoic Acid (C18:0)[b,c,d]	Stearic Acid	2.18 ± 0.32
(Z)-9-Octadecenoic Acid (C18:1 n-9)[b,c,d]	Oleic Acid	8.09 ± 0.40
(E)-9-Octadecenoic Acid (C18:1-9t)[c]	Elaidic Acid	0.052 ± 0.011
(Z)-11-Octadecenoic Acid (C18:1 n-7)[c]	Vaccenic Acid	0.552 ± 0.077
(E)-11-Octadecenoic Acid (C18:1-11t)[c]	*trans*-Vaccenic Acid	0.019 ± 0.010
Total *cis*-C18:1[c]		7.68 ± 0.15
Total *trans*-C18:1[c]		0.062 ± 0.010
(Z,Z)-9,12-Octadecadienoic Acid (C18:2 n-6)[b,c,d]	Linoleic Acid	3.32 ± 0.42
Total *trans*-C18:2[c]		0.0200 ± 0.0069
Total *cis*-C18:2[c]		2.96 ± 0.12
Total *trans*-C18:2 conjugated[c]		0.015 ± 0.012
(Z,Z,Z)-9,12,15-Octadecatrienoic Acid (C18:3 n-3)[b,c,d]	α-Linolenic Acid	0.133 ± 0.020
(Z,Z,Z)-6,9,12-Octadecatrienoic Acid (C18:3 n-6)[c]	γ-Linolenic Acid	0.0107 ± 0.0022
Eicosanoic Acid (C20:0)[b,c,d]	Arachidic Acid	0.0329 ± 0.0009
(Z)-11-Eicosenoic Acid (C20:1 n-9)[b,c,d]	Gondoic Acid	0.1322 ± 0.0044
Total *cis*-C20:1[c]		0.142 ± 0.014
(Z,Z)-11,14-Eicosadienoic Acid (C20:2 n-6)[c]		0.1250 ± 0.0095
(Z,Z,Z)-8,11,14-Eicosatrienoic Acid (C20:3 n-3)[c]	Dihomo-γ-linolenic Acid	0.0266 ± 0.0023
(Z,Z,Z)-11,14,17-Eicosatrienoic Acid (C20:3 n-3)[c]		0.0140 ± 0.0034
(Z)-13-Docosenoic Acid (C22:1 n-9)[c]	Erucic Acid	0.0230 ± 0.0025
Total *cis*-C22:4[c]		0.0325 ± 0.0035
Total *cis*-C22:5[c]		0.0140 ± 0.0012
Tetracosanoic Acid (C24:0)[b,c,d]	Lignoceric Acid	0.0068 ± 0.0003
(Z)-15-Tetracosenoic Acid (C24:1 n-9)[b,c,d]	Nervonic Acid	0.0228 ± 0.0009
Saturated Fat[c]		6.40 ± 0.15
cis-Monounsaturated Fat[c]		8.48 ± 0.24
cis-Polyunsaturated Fat[c]		3.293 ± 0.092
Total *trans* Fat[c]		0.088 ± 0.023
Total ω-3 Fatty Acids[c]		0.135 ± 0.015
Total ω-6 Fatty Acids[c]		3.127 ± 0.093

[a] These values are expressed as $x \pm 2u(x)$, where x is a mean value and $u(x)$ is its associated standard uncertainty. The method-specific value of the analyte lies within the interval $x \pm U_{95\%}(x)$ with 95 % confidence.
[b] NIST GC-MS
[c] Collaborating laboratories
[d] NIST GC-FID

Table A3. Non-Certified Values for Amino Acids in SRM 1546a

	Mass Fraction[a] (g/100 g)		
Alanine	0.94	±	0.06
Arginine	0.99	±	0.06
Aspartic Acid	1.4	±	0.2
Cystine	0.148	±	0.007
Glutamic Acid	2.2	±	0.3
Glycine	0.92	±	0.03
Histidine	0.52	±	0.03
Hydroxyproline	0.23	±	0.02
Isoleucine	0.6	±	0.1
Leucine	1.17	±	0.06
Lysine	1.23	±	0.04
Methionine	0.39	±	0.04
Phenylalanine	0.62	±	0.03
Proline	0.7	±	0.1
Serine	0.64	±	0.04
Threonine	0.68	±	0.07
Tryptophan	0.15	±	0.03
Tyrosine	0.49	±	0.04
Valine	0.68	±	0.05

[a] These values are expressed as $x \pm 2u(x)$, where x is a mean value and $u(x)$ is its associated standard uncertainty. The method-specific value of the analyte lies within the interval $x \pm U_{95\%}(x)$ with 95 % confidence.

Table A4. Non-Certified Values for Proximates and Calories in SRM 1546a

	Mass Fraction[a] (g/100 g)		
Solids	39.73	±	0.22
Ash	3.08	±	0.05
Protein	15.68	±	0.18
Carbohydrates	1.65	±	0.47
Fat (as the sum of fatty acids as triglycerides)	18.96	±	0.40

	Energy[a,b] (kcal per 100 g)		
Calories	242	±	4

[a] These values are expressed as $x \pm 2u(x)$, where x is a mean value and $u(x)$ is its associated standard uncertainty. The method-specific value of the analyte lies within the interval $x \pm U_{95\%}(x)$ with 95 % confidence.

[b] Calories were determined by collaborating laboratories as the median of lab mean caloric calculations from the interlaboratory comparison exercise. If the mean proximate values above are used for calculation, with caloric equivalents of 9, 4, and 4 for fat (as the sum of fatty acids as triglycerides), protein, and carbohydrates, respectively, the mean caloric content is 240 kcal per 100 grams.

Period of Validity: The non-certified values are valid within the measurement uncertainty specified until **31 January 2034**. The value assignments are nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

Maintenance of Non-Certified Values: NIST will monitor this material to the end of its period of validity. If substantive technical changes occur that affect the non-certified values during this period, NIST will update this Appendix and notify registered users. SRM users can register online from a link available on the NIST SRM website or fill out the user registration form that is supplied with the SRM. Registration will facilitate notification. Before making use of any of the values delivered by this material, users should verify they have the most recent version of this documentation, available through the NIST SRM website (https://www.nist.gov/srm).

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APPENDIX B

Methods Used in Value Assignment of SRM 1546a

Cholesterol: The mass fraction of cholesterol was measured using the ID-GC-MS method developed at NIST for serum cholesterol [4] and modified for the determination of cholesterol in food matrices using the approach in AOAC International Official Method 996.06 for hydrolysis [5]. One sample set consisted of single 2.0 g test portions from each of nine cans of SRM 1546a, weighed into Pyrex test tubes. The second sample set consisted of single 1.0 g test portions from each of ten cans of SRM 1546a, weighed into Pyrex test tubes. An aliquot of a solution containing a known mass of the internal standard, cholesterol-$^{13}C_3$, was added to each tube. Cholesterol esters were hydrolyzed by heating the samples in an alcohol-KOH solution for 1 h at 100 °C. Cholesterol was extracted into hexane, and a portion of the hexane extract was evaporated to dryness prior to addition of N,O-bis(trimethylsilyl)acetamide to convert cholesterol to the trimethylsilyl (TMS) derivative. GC-MS was performed using a 30 m phenyl:methyl polysiloxane (5:95 mole fraction) non-polar fused silica column directly interfaced to the ion source. Cholesterol was determined in the electron ionization mode with selected ion monitoring at *m/z* 458 and *m/z* 461 for the unlabeled and labeled cholesterol-TMS, respectively. Calibrants were prepared gravimetrically, from SRM 911c *Cholesterol*, at levels intended to approximate the level of the cholesterol in the SRM following extraction. A single internal standard solution was used for the calibrants and samples. Calculations are based on linear regression analysis for the calibrants.

Vitamins: Value assignment of the mass fractions of the vitamins in SRM 1546a was based on the combination of results of measurements from NIST and collaborating laboratories, where appropriate, as described in Table B1.

Table B1. Methods Used in Value Assignment for Vitamins in SRM 1546a

Vitamin	NIST Method	Method(s) Reported by Collaborating Laboratories
Thiamine (Vitamin B_1)	ID-LC-MS/MS [6]	Autoanalyzer; Fluorimetry; LC-FL; LC-MS; Microbiological assay
Riboflavin (Vitamin B_2)	ID-LC-MS/MS [6]	Autoanalyzer; LC-Abs; LC-FL; LC-MS; Microbiological assay
Niacin (Vitamin B_3)	ID-LC-MS/MS [6]	
Niacinamide (Vitamin B_3)	ID-LC-MS/MS [6]	
Total Vitamin B_3 as Niacinamide	ID-LC-MS/MS [6]	LC-FL; LC-MS; Microbiological assay
Total Vitamin B_{12} by Microbiological Assay		Microbiological assay
25-Hydroxyvitamin D_3	ID-LC-MS/MS	ID-LC-MS/MS [7]
Cholecalciferol (Vitamin D_3)		ID-LC-MS/MS [7]
Choline	ID-LC-MS [8]	
Carnitine	ID-LC-MS [8]	

ID-LC-MS: Isotope Dilution Liquid Chromatography with Mass Spectrometry; ID-LC-MS/MS: Isotope Dilution Liquid Chromatography with Tandem Mass Spectrometry; LC-Abs: liquid chromatography with absorbance detection; LC-FL: liquid chromatography with fluorescence detection

NIST Analyses for Vitamin D_3 and 25-Hydroxyvitamin D_3 Using ID-LC-MS/MS: Mass fractions were measured in duplicate 2.0 g to 3.0 g test portions taken from each of ten cans of SRM 1546a. Aliquots of internal standard solutions containing vitamin D_3-$^{13}C_5$ and 25-hydroxyvitamin D_3-$^{13}C_5$ were added to each calibrant and sample. Prior to extraction, the samples of SRM 1546a were incubated with lipase at 40 °C for 2 h to hydrolyze the fats. Ethanol containing butylated hydroxytoluene (BHT) and potassium carbonate was added to each sample, and the analytes and internal standards were extracted into hexane containing BHT by overnight stirring. The samples were centrifuged, the supernatants were decanted, and an additional aliquot of hexane containing BHT was added. Samples were extracted further by a combination of sonication and rotary mixing, then centrifuged, and the supernatants combined with those from the previous extraction. Two additional cycles of sonication and rotary mixing were conducted, for a total of four extractions. The pooled organic layers were dried under nitrogen to approximately 40 mL and magnesium sulfate was added. Following vortex mixing and centrifugation, the organic layer was decanted and evaporated to dryness under nitrogen. The analytes were derivatized with 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) and reconstituted in a mixture of methanol and ethyl acetate for analysis by positive-ion mode LC-MS/MS. A gradient method with a water/methanol mobile phase and a pentafluorophenyl column were used for LC-MS/MS determination. Vitamin D_3+PTAD and vitamin D_3-$^{13}C_5$+PTAD were measured at transitions *m/z* 560 → *m/z* 298 and *m/z* 565 → *m/z* 298, respectively. 25-Hydroxyvitamin D_3+PTAD and 25-hydroxyvitamin D_3-$^{13}C_5$+PTAD were

measured at transitions *m/z* 558 → *m/z* 298 and *m/z* 563 → *m/z* 298, respectively. Calibrants were prepared gravimetrically, at levels intended to approximate the levels of the vitamins in the SRM following extraction. The purity of the neat vitamin D_3 calibrant material was determined by the manufacturer and confirmed at NIST using spectrophotometry. The purity of the neat 25-hydroxyvitamin D_3 calibrant material was determined at NIST using LC-absorbance, Karl Fischer titration, thermogravimetric analysis, and qNMR. A single internal standard solution was used for the calibrants and samples.

Elements: Value assignment of the mass fractions of the elements in SRM 1546a was based on the combination of results of measurements from NIST and collaborating laboratories, where appropriate, as described in Table B2.

Table B2. Methods Used in Value Assignment for Elements in SRM 1546a

Element	NIST Method(s)	Method(s) Reported by Collaborating Laboratories
Barium (Ba)	ICP-MS; ICP-OES	
Boron (B)	PGAA	
Calcium (Ca)	ICP-OES	AAS; ICP-OES
Chlorine (Cl)	ICP-OES; INAA	
Copper (Cu)	ICP-MS; ICP-OES	AAS; ICP-MS; ICP-OES
Iron (Fe)	ICP-OES	AAS; ICP-MS; ICP-OES
Magnesium (Mg)	ICP-OES	AAS; ICP-MS; ICP-OES
Manganese (Mn)	ICP-MS; ICP-OES	AAS; ICP-MS; ICP-OES
Molybdenum (Mo)	ICP-MS	
Phosphorus (P)	ICP-OES	Colorimetry; ICP-MS; ICP-OES
Potassium (K)	ICP-OES	AAS; ICP-OES
Rubidium (Rb)	INAA	
Selenium (Se)	ICP-MS; INAA	
Sodium (Na)	ICP-OES; INAA	AAS; ICP-OES
Strontium (Sr)	ICP-MS; ICP-OES	
Zinc (Zn)	ICP-OES	AAS; ICP-MS; ICP-OES

AAS: atomic absorption spectroscopy; ICP-OES: Inductively Coupled Plasma Optical Emission Spectrometry; ICP-MS: Inductively Coupled Plasma Mass Spectrometry; INAA: Instrumental Neutron Activation Analysis; PGAA: Thermal Neutron Prompt Gamma ray Activation Analysis

NIST Analyses Using ICP-OES and/or ICP-MS: Duplicate 3.5 g to 4.0 g test portions were taken from each of 10 cans of SRM 1546a and were digested in a microwave sample preparation system using nitric acid or a nitric acid/hydrofluoric acid mixture. Quantitation for ICP-OES and ICP-MS was based on the method of standard additions using SRM 3100 series single element standard solutions.

NIST Analyses Using PGAA: Individual disks were prepared from 1 g test portions taken from each of six cans of SRM 1546a. Samples, controls, and standards, prepared from SRM 3107 *Boron (B) Standard Solution*, were packaged individually in clean Teflon bags and irradiated individually for less than 1 h. Gamma-ray spectra up to 11 MeV were collected, and the boron gamma-ray signal at 477 keV was monitored and compared to that of the standard to determine the mass fraction of boron.

NIST Analyses Using INAA: Individual disks were prepared from 0.4 g test portions taken from each of six cans of SRM 1546a. Samples, controls, and standards, prepared from SRM 3100 series single element standard solutions, were packaged individually in clean polyethylene bags and irradiated individually. For determination of chlorine, samples were irradiated at 20 MW for 60 s and nuclides were counted for 5 min after a 10 min decay. For determination of sodium, samples were irradiated at 20 MW for 60 s and nuclides were counted for 30 min after a 40 min decay. For determination of selenium and rubidium, samples were irradiated at 20 MW for 4 h and nuclides were counted for 8 h following a decay of several weeks.

Nitrate: Value assignment of the mass fraction of nitrate in SRM 1546a was based on the combination of measurements made by collaborating laboratories. Collaborating laboratories reporting nitrate data used ion chromatography, ion selective electrode, or LC-absorbance.

Fatty Acids: Value assignment of the mass fractions of fatty acids in SRM 1546a was based on the combination of measurements made using two extraction procedures and two different analytical methods at NIST and by collaborating laboratories, where appropriate. NIST provided results using gas chromatography (GC) with flame ionization detection (FID) and GC with mass spectrometric (MS) detection as described below. Collaborating laboratories did not provide information about methods used for fatty acids determination.

NIST Analyses Using GC-FID: The mass fractions were determined from two 1.5 g to 2.0 g test portions taken from each of 10 cans of SRM 1546a. The meat homogenate and internal standard solution (tricosanoic acid, palmitic acid-d_{35}, and myristic acid-d_{27}) were mixed with diatomaceous earth in pressurized fluid extraction (PFE) cells. Following PFE with hexane:dichloromethane:methanol (70:25:5 volume fraction) containing approximately 1 mg/g butylated hydroxytoluene (BHT), sodium sulfate was added to absorb excess water. Extracts were combined with methanolic (*m*-trifluoromethylphenyl) trimethylammonium hydroxide (1:1 volume fraction), vortexed, and allowed to stand for at least 30 min prior to analysis by GC-FID. GC-FID was performed using a 0.25 mm × 100 m biscyanopropyl polysiloxane fused silica capillary column. Calibrants were prepared gravimetrically from SRM 2377 *Fatty Acid Methyl Esters in 2,2,4-Trimethylpentane*, at levels intended to approximate the levels of the fatty acids in the SRM following extraction. A single internal standard solution was used for the calibrants and samples. Calculations are based on average response factors for the calibrants.

NIST Analyses Using GC-MS: The mass fractions were determined from one 1.5 g to 2.0 g test portion from each of six cans of SRM 1546a. The meat homogenate and internal standard solution (tricosanoic acid, palmitic acid-d_{35}, and myristic acid-d_{27}) were mixed with diatomaceous earth in PFE cells. Following PFE with hexane:acetone (80:20 volume fraction) containing approximately 1 mg/g BHT, sodium sulfate was added to absorb excess water. Extracts were combined with methanolic sodium hydroxide, blanketed with N_2, capped, mixed, and heated in a dry bath at 100 °C for 30 min with gentle shaking every 10 min. Extracts were cooled to 40 °C and fatty acids were extracted with 40 mg/L BHT in hexane and saturated aqueous sodium chloride solution. The hexane/BHT layer was removed and the hexane/BHT extraction repeated twice and combined with the first extracted portion. A subsample of the combined extracts was analyzed by GC-MS. GC-MS was performed using a 0.25 mm × 60 m fused silica capillary column containing a cyanopropyl:methylpolysiloxane (50:50 mole fraction) phase. Calibrants were prepared gravimetrically from SRM 2377 *Fatty Acid Methyl Esters in 2,2,4-Trimethylpentane*, at levels intended to approximate the levels of the fatty acids in the SRM following extraction. A single internal standard solution was used for the calibrants and samples. Calculations are based on average response factors for the calibrants.

Amino Acids: Value assignment of the mass fractions of amino acids in SRM 1546a was based on the combination of measurements made by collaborating laboratories. All collaborating laboratories reporting amino acids data used hydrolysis and derivatization followed by liquid chromatography.

Proximates: Value assignment of the mass fractions of proximates in SRM 1546a was based on the combination of measurements made by collaborating laboratories as described in Table B3. Collaborating laboratories did not provide information about methods used for fat determination.

Table B3. Methods Used in Value Assignment for Proximates in SRM 1546a

Proximate	Method(s) Reported by Collaborating Laboratories
Solids	Forced air oven drying; TGA; vacuum oven drying
Ash	Weight loss after ignition in muffle furnace; TGA
Protein	Calculated from nitrogen results (factor 6.25)
Carbohydrates	Calculated as solids – (protein + fat + ash)
Calories	Calculated as (9 x fat) + (4 x protein) + (4 x carbohydrate)

TGA: Thermogravimetric analysis

Collaborating Laboratories' Analyses: The GMA FIACSG laboratories were asked to use their usual methods to make single measurements of fatty acids, cholesterol, proximates, calories, elements, vitamins, and amino acids on test portions taken from each of two cans of SRM 1546a. Because of variability among data provided by laboratories participating in this interlaboratory comparison exercise, the median of laboratory means is used, with the uncertainty estimated using the median absolute deviation (MADe) [9]. The laboratories participating in the USDA interlaboratory study were asked to use their usual methods to make single measurements of vitamin D and metabolites on test portions taken from each of three cans of SRM 1546a [7]. The mean of laboratory means is used, with the uncertainty estimated using the standard error of the mean of laboratory means. The laboratories participating in the HAMQAP study were asked to use their usual methods to make single measurements of nitrate on test portions taken

from each of three cans of SRM 1546a [10]. The weighted median of laboratory means is used, with the uncertainty estimated using a bootstrap procedure based on a Laplace random effects model [4,11].

Homogeneity Assessment: The homogeneity of fatty acids, cholesterol, elements, and vitamins was assessed at NIST using the methods and test portion sizes described above. Analysis of the variance showed statistically significant heterogeneity in some cases, and the uncertainties for barium, calcium, pantothenic acid, pyridoxamine, pyridoxine, riboflavin, sodium, strontium, thiamine, and total vitamin B_6 as pyridoxine all incorporate an uncertainty component for possible heterogeneity. Homogeneity of constituents measured solely by collaborating laboratories (e.g., proximates, amino acids) was not assessed, although the data were treated as though these analytes were homogeneously distributed.

Value Assignment: For calculation of assigned values for analytes that were measured only by NIST, the mean of the mean values from NIST results was used. The collaborating laboratories reported the individual results for each of their analyses for a given analyte. The mean of each laboratory's results was then determined. For calculation of assigned values for analytes that were measured only by the GMA FIACSG laboratories or HAMQAP laboratories, the median or weighted median of the laboratory means was used. For analytes that were also measured by NIST, the mean of the individual sets of NIST data were averaged with the median of the individual GMA FIACSG laboratory means or the mean of the USDA laboratory means, as appropriate.

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APPENDIX C

Contributors to Value Assignment of SRM 1546a

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Conagra Foods (Omaha, NE)
Covance Laboratories, Inc. (Madison, WI)
Del Monte Foods (Walnut Creek, CA)
Eurofins Central Analytical Laboratories (Metairie, LA)
Eurofins Chemical Control (Cuneo, Italy)
Eurofins Nutrition Analysis Center (Des Moines, IA)
Eurofins Scientific Development (Nantes, France)
Eurofins Steins Laboratorium (Vejen, Denmark)
General Mills, Inc. (Golden Valley, MN)
Hormel Foods Corporation (Austin, MN)
Krueger Food Laboratories (Billerica, MA)
Land O'Lakes (Arden Hills, MN)
Mars Petcare (Kansas City, MO)
Nestle (Dublin, OH)
Schwan Food Company (Salina, KS)
Silliker Ibérica (Barcelona, Spain)
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