



National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1546a

Meat Homogenate

This Standard Reference Material (SRM) is intended primarily for validation of methods for determining fatty acids, cholesterol, proximates, calories, elements, vitamins, and amino acids in canned meat products and similar materials. This SRM can also be used for quality assurance when assigning values to in-house reference materials. The meat homogenate is a mixture of pork and chicken products blended together in a commercial process. A unit of SRM 1546a consists of four cans, each containing approximately 85 g of material.

Certified Mass Fraction Values: Certified mass fraction values for fatty acids, cholesterol, elements, and vitamins in SRM 1546a, reported on an as-received basis, are provided in Tables 1 through 4. 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]. Analyses for value assignment were performed by NIST and collaborating laboratories. Certified values were calculated as the mean of the mean values from NIST methods and the median or mean of the means of results provided by collaborating laboratories, where appropriate. The associated uncertainties are expressed at an approximately 95 % level of confidence [2–4].

Reference Mass Fraction Values: Reference mass fraction values for additional fatty acids, elements, and vitamins, proximates, calories, and amino acids in SRM 1546a, reported on an as-received basis, are provided in Tables 5 through 9. 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 [1] and is provided with an uncertainty 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. The reference mass fraction values were derived from results reported by NIST and/or collaborating laboratories.

Information Mass Fraction Values: An information mass fraction value for taurine is provided in Table 10. A NIST information value is a value that may be of interest to the SRM user, but insufficient information is available to assess the uncertainty associated with the value, therefore no uncertainty is provided [1]. Information values cannot be used to establish metrological traceability.

Expiration of Certification: The certification of **SRM 1546a** is valid, within the measurement uncertainty specified, until **31 January 2024**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see “Instructions for Storage and Use”). The 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.

Coordination of the technical measurements leading to the certification of this SRM was performed by M.M. Phillips and L.J. Wood of the NIST Chemical Sciences Division; K.E. Sharpless of the NIST Special Programs Office; D. Howell and W. Koshute of the Grocery Manufacturers Association (GMA, Washington, DC); and J. Roseland and K. Patterson of the United States Department of Agriculture (USDA, Beltsville, MD).

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Analytical measurements at NIST were performed by C.Q. Burdette, K.D. Chieh, G.E. Hahm, B.E. Lang, K.A. Lippa, J.L. Molloy, R. Oflaz, R.L. Paul, B.J. Porter, M.M. Phillips, M.M. Schantz, L.T. Sniegowski, M.J. Welch, and L.J. Wood of the NIST Chemical Sciences Division.

Analyses for value assignment were also performed by the following laboratories participating in a GMA Food Industry Analytical Chemists Share Group (FIACSG) interlaboratory comparison exercise: Campbell Soup (Camden, NJ); 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); Silliker Beijing (Beijing, China); Silliker Illinois Analytical Laboratory (Crete, IL); Silliker Ontario (Markham, ON Canada); The J.M. Smucker Co. (Orrville, OH); The National Food Laboratory (Livermore, CA). Additionally, the following laboratories participated in a USDA interlaboratory comparison evaluating methods for analysis of 25-hydroxyvitamin D₃ in foods: Covance Laboratories, Inc. (Madison, WI); Health Canada (Longueuil, QC, Canada); Heartland Laboratories (Ames, IA); Technical University of Denmark (Kongens Lyngby, Denmark).

Support for assignment of values for vitamin D₃ and 25-hydroxyvitamin D₃ was provided by National Institutes of Health, Office of Dietary Supplements (NIH-ODS). Technical consultation was provided by J.M. Betz (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.

NOTICE TO USERS: SRM 1546a IS INTENDED FOR RESEARCH USE; NOT FOR HUMAN CONSUMPTION.

INSTRUCTIONS FOR STORAGE AND USE

Storage: The SRM should be stored at room temperature or under refrigeration in the original unopened cans. The certification, which includes all value assignments, does not apply to contents of previously opened cans, because the stability of all analytes 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. To relate analytical determinations to the certified values in this Certificate of Analysis, the test portion mass indicated in the description of the NIST analyses for each group of analytes below should be used (see "Source, Preparation, and Analysis"). Results obtained in analyses should include their own estimates of uncertainty and can be compared to the listed values using procedures described in reference 5.

SOURCE, PREPARATION, AND ANALYSIS⁽¹⁾

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.

Analytical Approach for Determination of 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. Value assignment of the cholesterol mass fraction was based on measurements made by NIST using an isotope dilution (ID) GC-MS method.

NIST Analyses for Fatty Acids by GC-FID: The mass fractions of fatty acids were determined by GC-FID 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

⁽¹⁾ Certain commercial equipment, instruments, or materials are identified in this certificate 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.

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 for Fatty Acids by GC-MS: The mass fractions of fatty acids were determined by GC-MS 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 N₂, 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.

Analytical Approach for Determination of Cholesterol: The mass fraction of cholesterol was measured using the ID-GC-MS method developed at NIST for serum cholesterol [6] and modified for the determination of cholesterol in food matrices using the approach in AOAC International Official Method 996.06 for hydrolysis [7]. 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-¹³C₃, 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.

Analytical Approach for Determination of Elements: Value assignment of the mass fractions of the elements in SRM 1546a was based on the combination of results at NIST and collaborating laboratories, where appropriate. NIST provided measurements by using inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS), instrumental neutron activation analysis (INAA), and thermal neutron prompt gamma-ray activation analysis (PGAA).

NIST Analyses for Ba, Ca, Cl, Cu, Fe, K, Mg, Mn, Mo, Na, P, Se, Sr, and Zn Using ICP-OES and/or ICP-MS: The mass fractions of barium, calcium, chlorine, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, strontium, and zinc were measured by ICP-OES. Barium, copper, manganese, molybdenum, selenium, and strontium were measured by 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 for B Using PGAA: The mass fraction of boron was determined by PGAA from individual disks 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 for Cl, Na, Rb, and Se Using INAA: The mass fractions of chlorine, rubidium, selenium, and sodium were measured by INAA in individual disks that 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.

Analytical Approach for Determination of Vitamins: Value assignment of the mass fractions of the vitamins in SRM 1546a was based on the combination of results provided from NIST and collaborating laboratories, where appropriate. NIST provided measurements by using isotope dilution (ID) with liquid chromatography (LC) and mass spectrometry (MS) or tandem mass spectrometry (MS/MS).

NIST Analyses for Thiamine, Riboflavin, Niacinamide, Niacin, Pantothenic Acid, Pyridoxine, and Pyridoxamine: Mass fractions of thiamine, riboflavin, niacinamide, niacin, pantothenic acid, pyridoxine, and pyridoxamine were measured by ID-LC-MS/MS in duplicate 2 g test portions taken from each of ten cans of SRM 1546a. The analytes and internal standards were extracted into ammonium acetate at pH 2.6 for analysis by positive ion mode ID-LC-MS/MS. A gradient method with an ammonium formate buffer/methanol mobile phase and a C18 column were used for ID-LC-MS/MS determination of the vitamins. Calibrants were prepared gravimetrically at levels intended to approximate the levels of the vitamins in the SRM following extraction. The purity of neat calibrant materials was determined at NIST using LC-absorbance, Karl Fischer titration, thermogravimetric analysis, and differential scanning calorimetry. A single internal standard solution was used for the calibrants and samples. The analyte and internal standard transitions monitored for ID-LC-MS/MS are listed in Table 11.

NIST Analyses for Choline and Carnitine: Mass fractions of choline and carnitine were measured in duplicate 2.0 g test portions taken from each of ten cans of SRM 1546a. An aliquot of an internal standard solution containing $^2\text{H}_9$ -choline chloride and $^2\text{H}_9$ -carnitine hydrochloride was added to each calibrant and sample. The analytes and internal standards were extracted and hydrolyzed by microwave digestion in dilute hydrochloric acid for analysis by positive-ion mode LC-MS. A gradient method with an ammonium formate/acetonitrile mobile phase and a mixed-mode C18 column were used for LC-MS determination. Choline and $^2\text{H}_9$ -choline were measured at m/z 104 and m/z 113, respectively. Carnitine and $^2\text{H}_9$ -carnitine were measured at m/z 162 and m/z 171, respectively. Calibrants were prepared gravimetrically, at levels intended to approximate the levels of the vitamins in the SRM following extraction. The purity of neat calibrant materials was determined at NIST using quantitative proton nuclear magnetic resonance spectroscopy (qNMR). A single internal standard solution was used for the calibrants and samples.

NIST Analyses for Vitamin D₃ and 25-Hydroxyvitamin D₃: Mass fractions of vitamin D₃ (cholecalciferol) and 25-hydroxyvitamin D₃ 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 D₃- $^{13}\text{C}_5$ and 25-hydroxyvitamin D₃- $^{13}\text{C}_5$ 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 D₃+PTAD and vitamin D₃- $^{13}\text{C}_5$ +PTAD were measured at transitions m/z 560 \rightarrow m/z 298 and m/z 565 \rightarrow m/z 298, respectively. 25-Hydroxyvitamin D₃+PTAD and 25-hydroxyvitamin D₃- $^{13}\text{C}_5$ +PTAD were measured at transitions m/z 558 \rightarrow m/z 298 and m/z 563 \rightarrow 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 D₃ calibrant material was determined by the manufacturer and confirmed at NIST using spectrophotometry. The purity of the neat 25-hydroxyvitamin D₃ 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.

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) [8]. 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 [9]. The mean of laboratory means is used, with the uncertainty estimated using the standard error of the mean of laboratory means. The methods used by collaborating laboratories are indicated below.

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 B₆ 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, the 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.

Certified Mass Fraction Values for Fatty Acids as Free Fatty Acids: Each certified mass fraction value is the combined mean from the mean of NIST GC-MS data, the mean of NIST GC-FID data, and the median of the means of results provided by collaborating laboratories, where appropriate. The method reported by collaborating laboratories was GC-FID. 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 incorporates the observed difference between the results from the methods and their respective uncertainties consistent with the JCGM Guide and with its Supplement 1, and k is a coverage factor corresponding to approximately 95 % confidence [2–4]. The measurand is the total mass fraction for each fatty acid listed in Table 1 on an as-received basis. Metrological traceability is to the SI derived unit for mass fraction (expressed as grams per 100 grams).

Table 1. Certified Mass Fraction Values for Fatty Acids (as Free Fatty Acids) in SRM 1546a

	Common Name	Mass Fraction (g/100 g)	Coverage Factor, k
Decanoic Acid (C10:0) ^(a,b)	Capric Acid	0.0167 ± 0.0017	2.00
Dodecanoic Acid (C12:0) ^(a,b,c)	Lauric Acid	0.0153 ± 0.0011	2.00
Tetradecanoic Acid (C14:0) ^(a,b,c)	Myristic Acid	0.245 ± 0.023	2.00
(Z)-9-Tetradecenoic Acid (C14:1 n-5) ^(a,b,c)	Myristoleic Acid	0.0118 ± 0.0028	2.00
Hexadecanoic Acid (C16:0) ^(a,b,c)	Palmitic Acid	4.63 ± 0.53	2.00
(Z)-9-Hexadecenoic Acid (C16:1 n-7) ^(a,b,c)	Palmitoleic Acid	0.618 ± 0.078	2.00
Octadecanoic Acid (C18:0) ^(a,b,c)	Stearic Acid	2.18 ± 0.32	2.00
(Z)-9-Octadecenoic Acid (C18:1 n-9) ^(a,b,c)	Oleic Acid	8.09 ± 0.40	2.00
(Z)-11-Octadecenoic Acid (C18:1 n-7) ^(a,c)	Vaccenic Acid	0.324 ± 0.016	2.00
(Z,Z)-9,12-Octadecadienoic Acid (C18:2 n-6) ^(a,b,c)	Linoleic Acid	3.32 ± 0.42	2.00
(Z,Z,Z)-9,12,15-Octadecatrienoic Acid (C18:3 n-3) ^(a,b,c)	α -Linolenic Acid	0.133 ± 0.020	2.00
Eicosanoic Acid (C20:0) ^(a,b,c)	Arachidic Acid	0.0329 ± 0.0009	2.00
(Z)-11-Eicosenoic Acid (C20:1 n-9) ^(a,b,c)	Gondoic Acid	0.1322 ± 0.0044	2.00
(Z,Z,Z,Z)-5,8,11,14-Eicosatetraenoic Acid (C20:4 n-6) ^(a,c)	Arachidonic Acid	0.0201 ± 0.0011	2.00
Docosanoic Acid (C22:0) ^(a,c)	Behenic Acid	0.0442 ± 0.0010	2.00
Tetracosanoic Acid (C24:0) ^(a,b,c)	Lignoceric Acid	0.0068 ± 0.0003	2.00
(Z)-15-Tetracosenoic Acid (C24:1 n-9) ^(a,b,c)	Nervonic Acid	0.0228 ± 0.0009	2.00

^(a) NIST GC-MS

^(b) Collaborating laboratories

^(c) NIST GC-FID

Certified Mass Fraction Value for Cholesterol: The certified mass fraction for cholesterol is the mean of results obtained by NIST using ID-GC-MS. The uncertainty provided with the 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 represents the combined uncertainty consistent with the JCGM Guide and with its Supplement 1, and k is a coverage factor corresponding to approximately 95 % confidence [2,3]. The uncertainty for cholesterol incorporates Type B uncertainties for purity of the reference compound, completeness of hydrolysis, stability of cholesterol in base, and the difference between the certification set of data and a confirming set of data using a different GC column and different ions. The measurand is the total mass fraction for cholesterol as listed in Table 2 on an as-received basis. Metrological traceability is to the SI derived unit for mass fraction (expressed as milligrams per gram).

Table 2. Certified Mass Fraction Value for Cholesterol in SRM 1546a

	Mass Fraction (mg/g)	Coverage Factor, k
Cholesterol	0.717 ± 0.022	2.00

Certified Mass Fraction Values for Elements: Each certified mass fraction value is the combined mean from the mean of results from analyses by NIST and the median of the means of results provided by collaborating laboratories, where appropriate. 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 incorporates the observed difference between the results from the methods and their respective uncertainties consistent with the JCGM Guide and with its Supplement 1, and k is a coverage factor corresponding to approximately 95 % confidence [2–4]. The uncertainty for sodium also incorporates an additional uncertainty component for possible inhomogeneity. The measurand is the total mass fraction for each element listed in Table 3 on an as-received basis. Metrological traceability is to the SI derived unit for mass fraction (expressed as milligrams per kilogram).

Table 3. Certified Mass Fraction Values for Elements in SRM 1546a

	Mass Fraction (mg/kg)	Coverage Factor, k
Copper (Cu) ^(a,b,c)	0.605 ± 0.051	2.00
Iron (Fe) ^(a,c)	10.17 ± 0.35	2.00
Magnesium (Mg) ^(a,c)	178.1 ± 4.8	2.00
Manganese (Mn) ^(a,b,c)	0.286 ± 0.024	2.00
Phosphorus (P) ^(a,c)	1651 ± 32	2.00
Potassium (K) ^(a,c)	2490 ± 210	2.00
Selenium (Se) ^(b,c,d)	0.281 ± 0.017	2.00
Sodium (Na) ^(a,c,d)	9600 ± 1100	2.00
Zinc (Zn) ^(a,c)	17.88 ± 0.35	2.00

^(a) NIST ICP-OES

^(b) NIST ICP-MS

^(c) Collaborating laboratories reported methods that included atomic absorption spectroscopy (AAS), ICP-OES, ICP-MS, and colorimetry.

^(d) NIST INAA

Certified Mass Fraction Values for Vitamins: Each certified mass fraction value is the combined mean from the mean of results from analyses by NIST and the median or mean of the means of results provided by collaborating laboratories, where appropriate. 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 incorporates the observed difference between the results from the methods and their respective uncertainties consistent with the JCGM Guide and with its Supplement 1, and k is a coverage factor corresponding to approximately 95 % confidence [2–4]. The uncertainties for pantothenic acid and pyridoxamine also incorporate an additional uncertainty component for possible inhomogeneity. The measurand is the total mass fraction for each vitamin listed in Table 4 on an as-received basis. Metrological traceability is to the SI derived unit for mass fraction (expressed as milligrams per kilogram).

Table 4. Certified Mass Fraction Values for Vitamins in SRM 1546a

	Mass Fraction (mg/kg)	Coverage Factor, k
Niacin (Vitamin B ₃) ^(a,b)	0.401 ± 0.022	2.09
Niacinamide (Vitamin B ₃) ^(a,b)	38.18 ± 0.74	2.09
Total Vitamin B ₃ as Niacinamide ^(a,c,d)	41.0 ± 4.8	2.00
Pantothenic Acid (Vitamin B ₅) ^(a,b,e)	4.58 ± 0.59	2.00
Pyridoxamine (Vitamin B ₆) ^(a,b)	0.272 ± 0.054	2.00
25-Hydroxyvitamin D ₃ ^(a,f)	0.00090 ± 0.00012	2.00

^(a) NIST ID-LC-MS/MS

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

^(c) Collaborating laboratories reported methods that included microbiological assay and LC with fluorescence detection.

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

^(e) Collaborating laboratories reported methods that included microbiological assay and LC-MS.

^(f) Collaborating laboratories reported methods that included ID-LC-MS/MS [9].

Reference Mass Fraction Values for Fatty Acids as Free Fatty Acids: Each reference mass fraction value is the median of the means of results provided by collaborating laboratories. The method reported by collaborating laboratories was GC-FID. 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 represents the combined uncertainty, consistent with the JCGM Guide and with its Supplement 1, and k is a coverage factor corresponding to approximately 95 % confidence [2,3]. The measurand is the mass fraction for each fatty acid listed in Table 5, on an as-received basis, as determined by the methods indicated. Metrological traceability is to mass fraction (expressed as grams per 100 grams), as realized by the methods used.

Table 5. Reference Mass Fraction Values for Fatty Acids (as Free Fatty Acids) in SRM 1546a

	Common Name	Mass Fraction (g/100 g)	Coverage Factor, k
Pentadecanoic Acid (C15:0)		0.010 ± 0.002	2.00
Heptadecanoic Acid (C17:0)	Margaric Acid	0.0575 ± 0.0028	2.18
(Z)-10-Heptadecenoic Acid (C17:1 n-6)	Margaroleic Acid	0.0480 ± 0.0050	2.31
(E)-9-Octadecenoic Acid (C18:1-9t)	Elaidic Acid	0.052 ± 0.011	2.36
(E)-11-Octadecenoic Acid (C18:1-11t)	<i>trans</i> -Vaccenic Acid	0.019 ± 0.010	2.57
Total <i>trans</i> -C18:1		0.062 ± 0.010	2.20
Total <i>cis</i> -C18:1		7.68 ± 0.15	2.16
Total <i>trans</i> -C18:2		0.0200 ± 0.0069	2.45
Total <i>cis</i> -C18:2		2.96 ± 0.12	2.20
Total <i>trans</i> -C18:2 conjugated		0.015 ± 0.012	2.78
(Z,Z,Z)-6,9,12-Octadecatrienoic Acid (C18:3 n-6)	γ -Linolenic Acid	0.0107 ± 0.0022	3.18
Total <i>cis</i> -C20:1		0.142 ± 0.014	2.18
(Z,Z)-11,14-Eicosadienoic Acid (C20:2 n-6)		0.1250 ± 0.0095	2.18
(Z,Z,Z)-8,11,14-Eicosatrienoic Acid (C20:3 n-3)	Dihomo- γ -linolenic Acid, DGLA	0.0266 ± 0.0023	2.26
(Z,Z,Z)-11,14,17-Eicosatrienoic Acid (C20:3 n-3)		0.0140 ± 0.0034	2.45
(Z)-13-Docosenoic Acid (C22:1 n-9)	Erucic Acid	0.0230 ± 0.0025	2.23
Total <i>cis</i> -C22:4		0.0325 ± 0.0035	2.78
Total <i>cis</i> -C22:5		0.0140 ± 0.0012	2.78
Saturated Fat		6.40 ± 0.15	2.16
<i>cis</i> -Monounsaturated Fat		8.48 ± 0.24	2.16
<i>cis</i> -Polyunsaturated Fat		3.293 ± 0.092	2.16
Total <i>trans</i> Fat		0.088 ± 0.023	2.16
Total ω -3 Fatty Acids		0.135 ± 0.015	2.16
Total ω -6 Fatty Acids		3.127 ± 0.093	2.18

Reference Mass Fraction Values for Elements: Each reference mass fraction value is the combined mean from the mean of results from analyses by NIST and the median of the means of results provided by collaborating laboratories, where appropriate. The uncertainty provided 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 represents the combined uncertainty consistent with the JCGM Guide and with its Supplement 1, and k is a coverage factor corresponding to approximately 95 % confidence [2–4]. The uncertainties for barium, calcium, and strontium also incorporate an additional uncertainty component for possible inhomogeneity. The measurand is the mass fraction for each element listed in Table 6 on an as-received basis, as determined by the method indicated. Metrological traceability is to mass fraction (expressed as milligrams per kilogram), as realized by the methods used.

Table 6. Reference Mass Fraction Values for Elements in SRM 1546a

	Mass Fraction (mg/kg)		Coverage Factor, k
Barium (Ba) ^(a,b)	0.077 ±	0.019	2.00
Boron (B) ^(c)	0.306 ±	0.039	2.57
Calcium (Ca) ^(a,d)	360 ±	130	2.00
Chlorine (Cl) ^(a,e)	15300 ±	2300	2.00
Molybdenum (Mo) ^(b)	0.016 ±	0.002	2.07
Rubidium (Rb) ^(e)	2.56 ±	0.11	2.20
Strontium (Sr) ^(a,b)	0.305 ±	0.070	2.00

^(a) NIST ICP-OES

^(b) NIST ICP-MS

^(c) NIST PGAA

^(d) Collaborating laboratories reported methods that included AAS and ICP-OES.

^(e) NIST INAA

Reference Mass Fraction Values for Vitamins: Each reference mass fraction value is the mean from the combination of the mean results from NIST and the median or mean of the means of results provided by collaborating laboratories, where appropriate. The uncertainty provided 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 represents the combined uncertainty consistent with the JCGM Guide and with its Supplement 1, and k is a coverage factor corresponding to approximately 95 % confidence [2–4]. The uncertainties for thiamine, riboflavin, pyridoxine, and total vitamin B₆ as pyridoxine also incorporate an additional uncertainty component for possible inhomogeneity. The measurand is the mass fraction for each vitamin listed in Table 7, on an as-received basis, as determined by the method indicated. Metrological traceability is to mass fraction (expressed as milligrams per kilogram) as realized by the methods used.

Table 7. Reference Mass Fraction Values for Vitamins in SRM 1546a

	Mass Fraction (mg/kg)	Coverage Factor, k
Thiamine (Vitamin B ₁) ^(a,b,c,d)	0.90 ± 0.48	2.00
Riboflavin (Vitamin B ₂) ^(a,d)	0.35 ± 0.10	2.00
Total Vitamin B ₅ by Microbiological Assay ^(e)	6.4 ± 2.4	2.45
Pyridoxine (Vitamin B ₆) ^(a,d)	0.044 ± 0.012	2.00
Total Vitamin B ₆ as Pyridoxine ^(a,d,f)	0.318 ± 0.062	2.00
Total Vitamin B ₆ by Microbiological Assay ^(e)	1.83 ± 0.69	3.18
Total Vitamin B ₁₂ by Microbiological Assay ^(e)	0.0055 ± 0.0016	2.78
Cholecalciferol (Vitamin D ₃) ^(a,g)	0.00256 ± 0.00053	2.00
Choline ^(h)	536.4 ± 9.8	2.09
Carnitine ^(h)	92.0 ± 1.4	2.09

^(a) NIST ID-LC-MS/MS

^(b) Collaborating laboratories reported methods that included digestion with fluorescence detection, LC-fluorescence, LC-MS, and an autoanalyzer.

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

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

^(e) Collaborating laboratories.

^(f) NIST measured pyridoxamine and pyridoxine individually, and pyridoxamine was mathematically converted to pyridoxine by multiplication by the ratio of the relative molecular masses.

^(g) Collaborating laboratories reported methods that included ID-LC-MS/MS.

^(h) NIST ID-LC-MS

Reference Values for Proximates and Calories: Each reference value is the median of the means of results provided by collaborating laboratories. The uncertainty provided 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 represents the combined uncertainty consistent with the JCGM Guide and with its Supplement 1, and k is a coverage factor corresponding to approximately 95 % confidence [2,3]. For proximates, the measurands are the mass fractions listed in Table 8, on an as-received basis, as determined by the methods indicated. Metrological traceability is to mass fraction (expressed as grams per 100 grams), as realized by the methods used. For calories, the measurand is the caloric content (expressed as kilocalories per 100 grams), listed in Table 8, on an as-received basis as determined by the method indicated, and metrological traceability is to the scale realized by that method for energy.

Table 8. Reference Values for Proximates and Calories in SRM 1546a

	Mass Fraction (g/100 g)	Coverage Factor, k
Solids ^(a)	39.73 ± 0.22	2.10
Ash ^(b)	3.08 ± 0.05	2.10
Protein ^(c)	15.68 ± 0.18	2.09
Carbohydrates ^(d)	1.65 ± 0.47	2.10
Fat (as the sum of fatty acids as triglycerides)	18.96 ± 0.40	2.10
	Energy (kcal per 100 g)	Coverage Factor, k
Calories ^(e)	242 ± 4	2.11

^(a) Solids were determined by collaborating laboratories using drying in a forced-air oven, drying in a vacuum oven, and thermogravimetric analysis.

^(b) Ash was determined by collaborating laboratories using weight loss after ignition in a muffle furnace and thermogravimetric analysis.

^(c) Nitrogen was determined by collaborating laboratories using Kjeldahl and combustion (LECO). A factor of 6.25 was used to convert nitrogen results to protein.

^(d) Carbohydrates were determined by collaborating laboratories by difference (solids less the sum of protein, fat, and ash).

^(e) 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 carbohydrate, respectively, the mean caloric content is 240 kcal per 100 grams.

Reference Mass Fraction Values for Amino Acids: Each reference mass fraction value is the median of the means of results provided by collaborating laboratories using hydrolysis followed by derivatization and LC. The uncertainty provided 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 represents the combined uncertainty consistent with the JCGM Guide and with its Supplement 1, and k is a coverage factor corresponding to approximately 95 % confidence [2,3]. The measurand is the mass fraction of each amino acid listed in Table 9, on an as-received basis, as determined by the method indicated. Metrological traceability is to mass fraction (expressed as grams per 100 grams), as realized by the methods used.

Table 9. Reference Mass Fraction Values for Amino Acids in SRM 1546a

	Mass Fraction (g/100 g)	Coverage Factor, k
Alanine	0.94 ± 0.06	2.57
Arginine	0.99 ± 0.06	2.57
Aspartic Acid	1.4 ± 0.2	2.45
Cystine	0.148 ± 0.007	3.18
Glutamic Acid	2.2 ± 0.3	2.57
Glycine	0.92 ± 0.03	2.45
Histidine	0.52 ± 0.03	2.45
Hydroxyproline	0.23 ± 0.02	4.30
Isoleucine	0.6 ± 0.1	2.45
Leucine	1.17 ± 0.06	2.45
Lysine	1.23 ± 0.04	2.45
Methionine	0.39 ± 0.04	2.57
Phenylalanine	0.62 ± 0.03	2.45
Proline	0.7 ± 0.1	2.57
Serine	0.64 ± 0.04	2.45
Threonine	0.68 ± 0.07	2.45
Tryptophan	0.15 ± 0.03	3.18
Tyrosine	0.49 ± 0.04	2.45
Valine	0.68 ± 0.05	2.45

Information Mass Fraction Value for Taurine: The information mass fraction value for taurine, reported on an as-received basis, is the average of the means of duplicate results provided by two collaborating laboratories using hydrolysis followed by derivatization and LC. No uncertainty is provided because there is insufficient information available for its assessment.

Table 10. Information Mass Fraction Value for Taurine in SRM 1546a

	Mass Fraction (mg/kg)
Taurine	700

Table 11. LC-MS/MS Transitions Monitored for Vitamins

Compound	Precursor Ion (<i>m/z</i>)	→ Product Ion (<i>m/z</i>)	Internal Standard (IS)	IS Precursor Ion (<i>m/z</i>)	→ IS Product Ion (<i>m/z</i>)
Thiamine	266	42 123	¹³ C ₃ -Thiamine	269	42 123
Riboflavin	377	43 172 198 243	¹³ C ₄ , ¹⁵ N ₂ -Riboflavin	383	43 175 202 249
Niacinamide	123	53 78 80	² H ₄ -Niacinamide	127	56 81 84
Pantothenic Acid	220	41 43 72 90	¹³ C ₃ , ¹⁵ N-Pantothenic Acid	224	41 43 76 94
Pyridoxine	170	77 80 134 152	¹³ C ₄ -Pyridoxine	174	81 83 138 156
Pyridoxamine	169	77 134 152	² H ₃ -Pyridoxamine	172	79 136 155

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Certificate Revision History: **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 D₃; addition of reference values for vitamin D₃, carnitine, and rubidium; update of reference value for chlorine; update of value for choline from an information value to a reference value; conversion of vitamin B₆ values to the non-salt form; addition of method information; editorial changes); **02 May 2014** (Original certificate date).

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