National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1849

Infant/Adult Nutritional Formula

This Standard Reference Material (SRM) is intended primarily for validation of methods for determining proximates, fatty acids, vitamins, elements, amino acids, and nucleotides in infant and adult nutritional formulas and similar materials. This SRM can also be used for quality assurance when assigning values to in-house reference materials. The SRM is a milk-based, hybrid infant/adult nutritional powder prepared by a manufacturer of infant formula and adult nutritional products. A unit of SRM 1849 consists of 10 packets, each containing approximately 10 g of material.

Certified Concentration Values: The certified concentration values of selected fatty acids, elements, and vitamins in SRM 1849 are provided in Tables 1 through 3, respectively. 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, the U.S. Department of Agriculture (USDA), and collaborating laboratories. All certified values are calculated as the mean of the mean values from NIST methods, the mean of data provided by USDA, the median of the results provided by collaborating laboratories, and the mean provided by the manufacturer, where available. All values were combined without weighting. The associated uncertainties are expressed at the 95 % level of confidence [2-3]. Values are reported on an as-received (not dry-mass) basis in mass fraction units [4].

Reference Concentration Values: Reference concentration values are provided for additional fatty acids (Table 4); proximates, lactose monohydrate, cholesterol, and calories (Table 5); vitamins (Table 6); selenium (Table 7); amino acids (Table 8); and nucleotides (Table 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 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. The reference concentrations were derived from results reported by NIST, collaborating laboratories, or the manufacturer. Values are reported on an as-received (not dry-mass) basis in mass fraction units [4].

Information Concentration Values: An information concentration value for L-carnitine 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].

Expiration of Certification: The certification of **SRM 1849** is valid, within the measurement uncertainties specified, until **30 April 2014**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see "Notice and Warning to Users"). 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) will facilitate notification.

Coordination of the technical measurements leading to the certification of this SRM was performed by L.C. Sander, K.E. Sharpless, and S.A. Wise of the NIST Analytical Chemistry Division, I-P. Ho of the Grocery Manufacturers Association (GMA, Washington, DC), and M.K. Mountford of the International Formula Council (Atlanta, GA).

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See Certificate Revision History on Page 12

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Analytical measurements at NIST were performed by R.M. Lindstrom, B.C. Nelson, K.W. Phinney, K. Putzbach, C.A. Rimmer, M.M. Schantz, R.O. Spatz, J.B. Thomas, and L.J. Wood of the NIST Analytical Chemistry Division. Analyses at USDA (Beltsville, MD) were performed by R. Atkinson, W.C. Byrdwell, P. Chen, and R. Goldschmidt under the direction of W.R. Wolf. Analyses for value assignment were also performed by the laboratories listed in Appendix A, which includes participants in the GMA Food Industry Analytical Chemists Committee's (FIACC's) interlaboratory comparison exercise.

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

Support aspects involved with the certification and issuance of this SRM were coordinated through the NIST Measurement Services Division.

NOTICE AND WARNING TO USERS

Storage: The SRM should be stored at –80 °C or lower in the original unopened packets. The certification does not apply to contents of previously opened packets as the stability of all analytes has not been investigated.

Warning: For laboratory use only; not for human consumption.

Instructions for Use: Before use, the contents of the packet should be mixed thoroughly. For certified values to be valid, test portions of the following masses should be used: 0.5 g for fatty acid analysis, between 0.2 g and 2 g for elemental analysis, and between 1.0 g and 2.5 g for vitamin analysis. The stability of analytes in previously opened and stored packets has not been investigated.

SOURCE, PREPARATION, AND ANALYSIS[1]

Source and Preparation: The SRM is a milk-based hybrid infant/adult nutritional powder, prepared by a manufacturer of infant formula and adult nutritional products. A base liquid containing all constituents was conventionally heat processed, homogenized, and then spray-dried. The product was packaged into single-use nitrogen-flushed pouches, each containing 10 g of powder. The material was stored below 0 °C following packaging, and is stored at NIST at –80 °C to enhance long-term stability. This material contains some nutrients at levels not permitted in infant formula and *is not an infant formula*.

Analytical Approach for Determination of Fatty Acids: Value assignment of the concentrations of fatty acids in SRM 1849 was based on the combination of measurements made using two different analytical methods at NIST, and by collaborating laboratories and the manufacturer. NIST provided results using two different analytical methods: gas chromatography (GC) with flame ionization detection (FID) and mass spectrometry (MS) as described below.

NIST Analyses for Fatty Acids: Two 0.5 g test portions from each of 12 packets of SRM 1849 were combined with wet Hydromatrix (Varian, Palo Alto, CA) and transferred to a glass extraction thimble containing glass wool. An internal standard solution containing stearic-d_{35} acid, arachidic-d_{39} acid, and myristic-d_{27} acid was added, and samples were extracted for 22 h using a 4 + 1 hexane + acetone solution (for GC/MS analysis) or 2 + 1 chloroform + methanol solution (for GC-FID analysis). Following extraction, extracts were concentrated in benzene, and 0.25 mL of MethPrep II (0.1 mol/L methanolic [*m*-trifluoromethylphenyl] trimethylammonium hydroxide, Alltech, Deerfield, IL) was added. Samples were mixed for 1 min and allowed to sit for at least 1 h prior to analysis by GC/MS or GC-FID. Six independently prepared calibrants were used for each of the methods. GC-FID was performed using a 0.25 mm × 100 m biscyanopropyl polysiloxane fused silica capillary column. GC/MS was performed using a 0.25 mm × 60 m fused silica capillary column containing a 50 % cyanopropyl + 50 % phenylpolysiloxane (mole fraction) phase. Calibrants were prepared gravimetrically, at levels intended to approximate the levels of the fatty acids in the SRM. A single internal standard solution was used for the calibrants and samples. Calculations are based on average response factors for the calibrants.

[1]Certain commercial equipment, instruments, or materials are identified in this certificate in order to specify adequately 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.

Analytical Approach for Determination of Vitamins: Value assignment of the concentrations of the vitamins in SRM 1849 was based on the combination of results provided from several different analytical methods at NIST, USDA, collaborating laboratories, and the manufacturer. NIST provided measurements by using a combination of different liquid chromatography (LC) methods with different detection as described below.

NIST Analyses for Fat-Soluble Vitamins: Vitamin A was measured by using combinations of two LC methods with absorbance or MS detection. Tocopherols, including α-tocopheryl acetate, were measured using two LC methods with absorbance or fluorescence detection. Vitamins D and K (cholecalciferol and phylloquinone, respectively) were measured at NIST using only LC/MS. Calibrants were prepared gravimetrically, at levels intended to approximate the levels of the fat-soluble vitamins in the SRM. Internal standards were employed; a single solution was used for the calibrants and samples.

β-Carotene was measured at NIST by using two LC methods with absorbance detection. Results from all sources, including collaborating laboratories, ranged from 2 μg/g to 12 μg/g, therefore a value could not be assigned.

Retinyl Palmitate, Tocopherols, α-Tocopheryl Acetate, Cholecalciferol, and Phylloquinone: Single 1.5 g to 2.5 g test portions of powder from each of three packets were accurately weighed into 50 mL polyethylene centrifuge tubes. Duplicate test portions were taken from each of three other packets. Separate aliquots from each of three internal standard solutions containing retinyl palmitate-d_4, vitamin K_1-d_4, and vitamin D_3-d_3 were added. Analytes were extracted into ethyl acetate by sonication and then mixing/rotation overnight. Four additional extractions were performed using sonication and 30 min of mixing/rotation. The supernatants for the individual test portions were combined and were evaporated to approximately 10 mL under nitrogen. They were injected without additional processing. Separations were performed on a C_{18} column with an isocratic mobile phase of 40 % methanol and 60 % acetonitrile containing 5 mmol/L ammonium acetate. The separation was monitored using an absorbance detector at 287 nm, but MS was used for quantitation. Retinyl palmitate and retinyl palmitate-d_4 were monitored together at *m/z* of 269 and 273, respectively. Vitamins K_1 and K_1-d_4 were monitored at *m/z* of 452 and 456, respectively. Vitamins D_3 and D_3-d_3 were monitored together at *m/z* of 385 and 388, respectively. Retinyl palmitate was measured at NIST in single test portions taken from each of six packets using this same method several months prior to the analyses in which all four fat-soluble vitamins were measured, and these data were also used for value assignment.

For measurement of the tocopherols and α-tocopheryl acetate, two 2.0 g to 2.5 g test portions from each of six packets were dissolved in about 5 g of sub-boiling HPLC-grade water and an internal standard (tocol) was added. The mixture was sonicated for 30 min at 30 °C prior to being extracted into ethyl acetate overnight on a rotating shaker. Subsequent extractions using the rotating shaker were performed for about 2 h each. Each sample was extracted at least 4 times or until the supernatant was colorless, not yellow. The supernatants from each extraction were combined and evaporated, and the residue was dissolved in ethyl acetate/ethanol containing 30 μg/mL butylated hydroxytoluene (an antioxidant) prior to LC analysis using a polymeric C_{18} column and a methanol/water/butanol gradient with absorbance detection at 292 nm. Retinyl palmitate was measured at NIST in duplicate test portions taken from each of six packets using this same method several months prior to the analyses in which the tocopherols and α-tocopheryl acetate were measured, and these data were also used for value assignment. The individual results for retinyl palmitate were converted to retinol equivalents using the relative molecular masses of the two compounds.

The tocopherols were also measured by using LC with fluorescence detection. Single 1.1 g test portions were removed from each of six packets and were combined gravimetrically with a solution of tocol (internal standard). The moistened powder was then dissolved in water. Potassium oxalate, ethanol, *tert*-butyl methyl ether, and petroleum ether were added, and analytes were extracted by shaking. The extraction was repeated, and the supernatants were combined and evaporated. The residue was reconstituted in ethyl acetate and analyzed without further processing. The separation was performed using a C_{30} column held at 5 °C and a mobile phase of 99 % methanol and 1 % water. Fluorescence detection of tocopherols was performed using an excitation wavelength of 295 nm and an emission wavelength of 330 nm. Absorbance of α-tocopheryl acetate was measured at 284 nm. The individual results for α-tocopheryl acetate were converted to α-tocopherol equivalents, which were then added to the α-tocopherol measured using fluorescence detection to calculate total α-tocopherol equivalents.

NIST Analyses for Water-Soluble Vitamins: Water-soluble vitamins were measured by using combinations of two LC methods with absorbance detection, MS, or MS/MS. Calibrants were prepared gravimetrically, at levels intended to approximate the levels of the vitamins in the SRM. In cases where an internal standard was employed, a single solution was used for the calibrants and samples.

Vitamins B_1, B_2, B_6, Niacinamide, and Pantothenic Acid: Vitamins B_1, B_2, B_6, niacinamide, and pantothenic acid were measured by LC/MS in duplicate 2 g test portions taken from each of six packets. Four internal standards were added: $^{13}C_3$-thiamine chloride; $^{2}H_4$-niacinamide; calcium $^{13}C_3$,^{15}N-pantothenate; and $^{13}C_4$-pyridoxine hydrochloride. The analytes and internal standards were extracted into dilute acetic acid for analysis by positive-ion mode LC/MS. A gradient method with an ammonium formate buffer/methanol mobile phase and a C_{18} column were used for LC/MS determination of vitamins B_1, B_2, and B_6, niacinamide, and pantothenic acid. Thiamine and $^{13}C_3$-thiamine were measured at *m/z* 265 and *m/z* 268, respectively. Niacinamide and $^{2}H_4$-niacinamide were measured at *m/z* 123 and *m/z* 127, respectively. Pantothenic acid and $^{13}C_3$,^{15}N-pantothenic acid were measured at *m/z* 220 and *m/z* 224, respectively. Pyridoxine and $^{13}C_4$-pyridoxine were measured at *m/z* 170 and *m/z* 174, respectively. Riboflavin was measured at *m/z* 377, with $^{13}C_4$-pyridoxine as the internal standard. Niacinamide was measured at NIST in duplicate test portions taken from each of six packets using this same method several months prior to the analyses in which the other water-soluble vitamins were measured, and these data were also used for value assignment.

Vitamins B_1, B_2, B_6, and niacinamide were measured in single 2.5 g test portions taken from each of six packets. The powder was dissolved in water and 4-pyridoxic acid (internal standard) was added along with metaphosphoric acid and acetonitrile. This mixture was sonicated and centrifuged prior to analysis using a C_{18} column held at 26 °C. A gradient of potassium phosphate/phosphoric acid and acetonitrile was used. Absorbance of vitamins B_1 and niacinamide was measured at 260 nm; absorbance of vitamin B_2 was measured at 266 nm. Fluorescence of vitamin B_6 was measured using an excitation wavelength of 269 nm and an emission wavelength of 520 nm.

Folic Acid: Folic acid measurements were made on two 1.0 g test portions taken from each of six packets. An internal standard, $^{13}C_5$-folic acid, was added. Water containing dithiothreitol and ammonium hydroxide was added to the samples that were analyzed by negative-ion mode LC/MS/MS; a phosphate buffer containing trifluoroacetic acid and ascorbic acid was added to the samples that were analyzed by positive-ion mode LC/MS/MS. Folic acid and $^{13}C_5$-folic acid were extracted on solid-phase extraction cartridges, from which they were eluted with a water/methanol solution containing ascorbic acid and formic acid. A gradient LC method with a water/methanol/acetic acid mobile phase and a pentafluorophenyl column were used for the negative-ion mode LC/MS/MS determination. The transitions at *m/z* 440 → *m/z* 311 and *m/z* 445 → *m/z* 311 were monitored. A gradient LC method with a water/methanol/formic acid mobile phase and a pentafluorophenyl column were used for the positive-ion mode LC/MS/MS determination. The transitions at *m/z* 442 → *m/z* 295 and *m/z* 447 → *m/z* 295 were monitored.

Biotin: Biotin was also measured in two 1.0 g test portions taken from each of six packets. $^{2}H_2$-biotin was added as an internal standard. Ammonium hydroxide was added to the samples that were analyzed by negative-ion mode LC/MS; formic acid was added to the samples that were analyzed by positive-ion LC/MS/MS. Biotin and $^{2}H_2$-biotin were extracted using solid-phase extraction cartridges for positive-ion LC/MS/MS analysis. Samples analyzed by negative-ion LC/MS were simply filtered. An isocratic LC method with a water/methanol/formic acid mobile phase and a C_{18} column were used for the negative-ion LC/MS determination. Biotin and $^{2}H_2$-biotin were measured at *m/z* 243 and *m/z* 245, respectively. An isocratic LC method with a water/methanol/formic acid mobile phase and a C_{18} column were used for the positive-ion LC/MS/MS determination. The transitions at *m/z* 245 → *m/z* 227 and *m/z* 247 → *m/z* 229 were monitored.

Analytical Approach for Determination of Elements: Value assignment of the concentrations of the elements in SRM 1849 was based on the combination of measurements from two different analytical methods at NIST, collaborating laboratories, and the manufacturer, where available. NIST provided measurements by using instrumental neutron activation analysis (INAA) and inductively coupled plasma optical emission spectrometry (ICP-OES). Collaborating laboratories used their usual methods (see Appendix B).

NIST Analyses for Elements: For the determination of calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc by ICP-OES, duplicate 0.5 g test portions were taken from each of six packets of SRM 1849 and were digested in nitric acid in a microwave oven. For the determination of chromium and molybdenum, duplicate 2.0 g portions were taken from each of six packets and were digested in nitric acid on a hot plate. Quantitation for both groups of analytes was based on the method of standard additions.

For the determination of chlorine, iodine, and sodium by INAA, individual disks were prepared from 0.2 g test portions taken from each of six packets of SRM 1849. Standards were prepared from compounds of known purity. Samples, standards, and controls were packaged individually in clean polyethylene bags and irradiated individually at 20 MW for 60 s. Nuclides were counted for 5 min after 5 min decay, for 10 min following 10 min decay, and for 20 min after a few hours decay.

Collaborating Laboratories' Analyses: The GMA FIACC laboratories (Appendix A) and a group of other laboratories were asked to use their usual methods to make single measurements on test portions taken from each of two or three packets of SRM 1849, respectively. The manufacturer of the material also provided data. The collaborating laboratories' data were combined with NIST data for calculation of the certified values. Collaborating laboratories' data alone were used to assign reference and information values for proximates, amino acids, nucleotides, ascorbic acid, vitamin B_{12}, choline, inositol, and carnitine. A summary of the methodological information and the number of laboratories using a particular analytical technique is provided in Appendix B.

Homogeneity Assessment: The homogeneity of elements, fatty acids, and vitamins was assessed at NIST using the methods and test portion sizes described above; analysis of variance did not show statistically significant heterogeneity. All analytes have been treated as though they are homogeneously distributed in the material although the homogeneity of the other analytes was not assessed.

Value Assignment: The laboratories listed in Appendix A 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 collaborating laboratories and/or the manufacturer, the median of the laboratory means and the mean of the manufacturer's data were averaged. (The standard deviation of the median was estimated using a robust estimate based on the median absolute deviation.) For analytes that were also measured by NIST, the mean of individual sets of USDA data (where available), the median of the individual collaborating laboratory means, the manufacturer's mean, and the mean of the individual sets of NIST data were averaged.

Table 1. Certified Concentrations for Fatty Acids as Triglycerides[a]

	Mass Fraction (%)			*k*
Octanoic Acid (C8:0)[b,c,d] (Caprylic Acid)	0.638	±	0.067	2.09
Decanoic Acid (C10:0)[b,c] (Capric Acid)	0.473	±	0.019	2.36
Dodecanoic Acid (C12:0)[b,c] (Lauric Acid)	3.712	±	0.075	2.08
Tetradecanoic Acid (C14:0)[b,c] (Myristic Acid)	1.521	±	0.021	2.07
Pentadecanoic Acid (C15:0)[b,c]	0.0070	±	0.0003	2.23
Hexadecanoic Acid (C16:0)[b,c] (Palmitic Acid)	2.50	±	0.16	2.57
(Z)-9-Hexadecenoic Acid (C16:1 n-7)[b,c] (Palmitoleic Acid)	0.0262	±	0.0016	2.11
Octadecanoic Acid (C18:0)[b,c] (Stearic Acid)	0.905	±	0.056	2.78
(Z)-9-Octadecenoic Acid[b,c] (C18:1 n-9) (Oleic Acid)	10.63	±	0.88	2.78
(Z)-11-Octadecenoic Acid (C18:1 n-7)[b,c] (Vaccenic Acid)	0.203	±	0.021	2.36
(Z,Z)-9,12-Octadecadienoic Acid (C18:2 n-6)[b,c,d] (Linoleic Acid)	6.02	±	0.10	2.06
(Z,Z,Z)-9,12,15-Octadecatrienoic Acid (C18:3 n-3)[b,c,d] (α-Linolenic Acid)	0.561	±	0.043	2.78
Eicosanoic Acid (C20:0)[b,c] (Arachidic Acid)	0.095	±	0.003	2.31
(Z,Z,Z,Z)-5,8,11,14-Eicosatetraenoic Acid (C20:4 n-6)[b,c,d] (Arachidonic Acid)	0.206	±	0.022	2.20
(Z,Z,Z,Z,Z,Z)-4,7,10,13,16,19-Docosahexaenoic Acid (C22:6)[b,c,d]	0.067	±	0.006	2.57
Tetracosanoic Acid (C24:0)[b,c] (Lignoceric Acid)	0.039	±	0.003	2.23

[a] Each certified concentration value, expressed as a mass fraction for the material as received, is the mean from the combination of the mean from each set of results from analyses by NIST using GC/MS and GC-FID, the median of the results provided by collaborating laboratories, and the mean result provided by the material manufacturer, where available. The uncertainty in the certified concentration, calculated according to the method described in the ISO Guide [2,3], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory and within-laboratory components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence level for each analyte. Analytical methodology information, including the number of collaborating laboratories whose data were used for value assignment, is provided in Appendix B.

[b] NIST GC/MS and GC-FID

[c] Collaborating laboratories (see Appendix A)

[d] Manufacturer

Table 2. Certified Concentrations for Selected Elements[a]

	Mass Fraction (mg/kg)			*k*
Calcium[b,c,d]	4900	±	130	2.57
Chlorine[c,d,e]	6280	±	140	2.57
Copper[b,c,d]	20.29	±	0.43	2.31
Chromium[b,c]	1.09	±	0.21	2.78
Iodine[c,e]	1.37	±	0.41	2.45
Iron[b,c,d]	177.1	±	3.3	2.20
Magnesium[b,c,d]	1578	±	69	2.57
Manganese[b,c,d]	51.00	±	0.53	2.20
Molybdenum[b,c]	1.62	±	0.15	2.78
Phosphorus[b,c,d]	3782	±	36	2.12
Potassium[b,c,d]	8860	±	130	2.45
Sodium[b,c,d,e]	4150	±	140	2.36
Zinc[b,c,d]	152.3	±	5.1	2.45

[a] Each certified concentration value, expressed as a mass fraction for the material as received, is the mean from the combination of the mean of results from analyses by NIST, the median of the results provided by collaborating laboratories, and the mean result provided by the material manufacturer, where available. The uncertainty in the certified concentration, calculated according to the method described in the ISO Guide [2,3], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory and within-laboratory components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence level for each analyte. Analytical methodology, including the number of collaborating laboratories whose data were used for value assignment, is provided in Appendix B.

[b] NIST ICP-OES

[c] Collaborating laboratories (see Appendix A)

[d] Manufacturer

[e] NIST INAA

Table 3. Certified Concentrations for Selected Vitamins[a]

	Mass Fraction (mg/kg)			*k*
Retinol (Vitamin A)[b,c,d,e,f]	16.4	±	1.3	2.78
Cholecalciferol (Vitamin D_3)[b,d,e,g,h]	0.251	±	0.027	2.78
α-Tocopherol[c,d,i,j]	369	±	16	2.57
γ-Tocopherol[c,d,j]	189	±	13	2.06
δ-Tocopherol[c,]	79.2	±	2.4	2.02
β-Tocopherol[e,j]	5.77	±	0.79	2.57
Phylloquinone (Vitamin K_1)[b,d,e,k]	2.20	±	0.18	2.45
Thiamine (Vitamin B_1) Hydrochloride[b,c,d,e,g,l,m]	15.8	±	1.3	2.57
Riboflavin (Vitamin B_2)[b,c,d,e,g,m,n]	17.4	±	1.0	2.57
Niacinamide[b,c,d,e,g,l,m]	97.5	±	2.3	2.45
Pantothenic Acid[b,g,m]	64.8	±	2.2	2.45
Pyridoxine (Vitamin B_6) Hydrochloride[b,c,d,e,g,m,n]	14.2	±	1.5	2.57
Folic Acid[d,e,g,m,o]	2.11	±	0.13	2.78
Biotin[b,d,e,g,o]	1.92	±	0.25	2.78

[a] Each certified concentration value, expressed as a mass fraction for the material as received, is the mean from the combination of the mean results from each set of analyses by NIST, the median of the results provided by collaborating laboratories, and the mean result provided by the material manufacturer, where available. The uncertainty in the certified concentration, calculated according to the method described in the ISO Guide [2,3], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory and within-laboratory components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence level for each analyte. Analytical methodology information, including the number of collaborating laboratories whose data were used for value assignment, is provided in Appendix B.

[b] NIST LC/MS

[c] NIST LC/absorbance

[d] Collaborating laboratories (see Appendix A)

[e] Manufacturer

[f] Retinol was added to SRM 1849 as retinyl palmitate. The certified value is expressed as retinol equivalents, and represents total (*cis* + *trans*) retinol.

[g] USDA LC/MS

[h] Some of the collaborating laboratories included pre-vitamin D in their vitamin D measurements, however results were indistinguishable from results for just vitamin D_3, therefore results were combined for value assignment.

[i] α-Tocopherol was added to SRM 1849 as RRR-α-tocopheryl acetate. The certified value is expressed as α-tocopherol equivalents and includes "naturally occurring" α-tocopherol as well as the acetate. The α-tocopherol content, excluding α-tocopheryl acetate, is about 50 mg/kg.

[j] NIST LC/fluorescence

[k] The manufacturer reported a value for *trans*-vitamin K; results were indistinguishable from the range of results reported for total vitamin K, and results were combined for value assignment.

[l] USDA LC/absorbance

[m] Collaborating laboratories reported measuring either total or free analyte; results were indistinguishable and have been combined for value assignment.

[n] USDA LC/fluorescence

[o] NIST LC/MS/MS

Table 4. Reference Concentrations for Selected Fatty Acids as Triglycerides[(a)]

	Mass Fraction (%)			*k*
Hexanoic Acid (C6:0) (Caproic Acid)	0.061	±	0.011	2.20
Heptadecanoic Acid (C17:0) (Margaric Acid)	0.015	±	0.001	2.20
(Z)-11-Eicosenoic Acid (C20:1 n-9) (Gondoic Acid)	0.062	±	0.007	2.31
(Z,Z,Z)-8,11,14-Eicosatrienoic Acid (C20:3)	0.020	±	0.004	2.31
Docosanoic Acid (C22:0) (Behenic Acid)	0.080	±	0.007	2.14
(Z)-15-Tetracosenoic Acid (C24:1 n-9) (Nervonic Acid)	0.024	±	0.004	2.31

[(a)] Each reference concentration value, expressed as a mass fraction for the material as received, is the median of the results provided by collaborating laboratories. The uncertainty in the reference concentration, calculated according to the method described in the ISO Guide [2,3], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory and within-laboratory components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence level for each analyte. Analytical methodology information, including the number of collaborating laboratories whose data were used for value assignment, is provided in Appendix B.

Table 5. Reference Concentrations for Proximates, Cholesterol, Lactose Monohydrate, and Calories[(a)]

	Mass Fraction (%)			*k*
Solids	98.4	±	0.1	2.09
Ash	4.52	±	0.04	2.16
Fat	31.0	±	0.6	2.31
Protein	13.3	±	0.1	2.45
Carbohydrates	50.2	±	0.3	2.13
Lactose Monohydrate	49.8	±	1.8	2.00
	Mass Fraction (mg/g)			*k*
Cholesterol	0.127	±	0.015	2.26
Calories[(b)]	527	±	4 kcal/100 g	2.13

[(a)] Each reference concentration value, expressed as a mass fraction for the material as received, is the median of the mean values provided by collaborating laboratories and the mean result provided by the material manufacturer, where available. The uncertainty in the reference concentration, calculated according to the method described in the ISO Guide [2,3], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory and within-laboratory components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence level for each analyte. Analytical methodology information, including the number of collaborating laboratories whose data were used for value assignment, is provided in Appendix B.

[(b)] The value for calories is the mean of individual 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, protein, and carbohydrate, respectively, the mean caloric content is 532.7 kcal/100 g.

Table 6. Reference Concentrations for Selected Vitamins[a]

	Mass Fraction (mg/kg)			*k*
Ascorbic Acid[b,c,d]	1060	±	30	2.09
Vitamin B_{12}[b,c]	0.040	±	0.008	2.45
Choline Ion[b,c]	882	±	88	2.36
Myo-Inositol[b,c]	398	±	26	2.57

[a] Each reference concentration value, expressed as a mass fraction for the material as received, is the mean from the combination of the median of the results provided by collaborating laboratories and the mean result provided by the material manufacturer, where available. The uncertainty in the reference concentration, calculated according to the method described in the ISO Guide [2,3], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory and within-laboratory components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence level for each analyte. Analytical methodology information, including the number of collaborating laboratories whose data were used for value assignment, is provided in Appendix B.

[b] Collaborating laboratories (see Appendix A)

[c] Manufacturer

[d] Does not include dehydroascorbic acid

Table 7. Reference Concentration for Selenium[a]

	Mass Fraction (mg/kg)			*k*
Selenium	0.889	±	0.057	2.26

[a] The reference concentration value, expressed as a mass fraction for the material as received, is the mean from the combination of the median of the results provided by collaborating laboratories and the mean result provided by the material manufacturer. The uncertainty in the reference concentration, calculated according to the method described in the ISO Guide [2,3], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory and within-laboratory components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence level for each analyte. Analytical methodology information, including the number of collaborating laboratories whose data were used for value assignment, is provided in Appendix B.

Table 8. Reference Concentrations for Amino Acids and Taurine[a]

	Mass Fraction (%)			*k*
Alanine	0.488	±	0.011	2.26
Arginine	0.411	±	0.024	2.36
Aspartic Acid	1.11	±	0.05	2.26
Cystine	0.143	±	0.012	2.36
Glutamic Acid	2.78	±	0.14	2.23
Glycine	0.250	±	0.007	2.36
Histidine	0.316	±	0.015	2.23
Isoleucine	0.688	±	0.043	2.31
Leucine	1.31	±	0.06	2.31
Lysine	1.05	±	0.03	2.31
Methionine	0.457	±	0.070	2.31
Phenylalanine	0.589	±	0.013	2.20
Proline	1.21	±	0.06	2.26
Serine	0.726	±	0.054	2.23
Taurine	0.035	±	0.007	2.57
Threonine	0.636	±	0.036	2.45
Tryptophan	0.188	±	0.015	2.36
Tyrosine	0.516	±	0.071	2.45
Valine	0.798	±	0.041	2.31

[a] Each reference concentration value, expressed as a mass fraction for the material as received, is the mean from the combination of the median of the results provided by collaborating laboratories and the mean result provided by the material manufacturer, where available. The uncertainty in the reference concentration, calculated according to the method described in the ISO Guide [2,3], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory and within-laboratory components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence level for each analyte. Analytical methodology information, including the number of collaborating laboratories whose data were used for value assignment, is provided in Appendix B.

Table 9. Reference Concentrations for Nucleotides[a]

	Mass Fraction (mg/kg)			*k*
Adenosine Monophosphate	106	±	5	2.57
Cytidine Monophosphate	305	±	5	2.31
Guanosine Monophosphate	147	±	38	4.30
Uridine Monophosphate	148	±	8	4.30

[a] Each reference concentration value, expressed as a mass fraction for the material as received, is the mean from the combination of the median of the results provided by collaborating laboratories and the mean result provided by the material manufacturer, where available. The uncertainty in the reference concentration, calculated according to the method described in the ISO Guide [2,3], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory and within-laboratory components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence level for each analyte. Analytical methodology information, including the number of collaborating laboratories whose data were used for value assignment, is provided in Appendix B.

Table 10. Information Concentration for L-Carnitine[(a)]

	Mass Fraction (mg/kg)
Free L-Carnitine	85

[(a)] The information concentration value, expressed as a mass fraction of carnitine in the material as received, is the mean result provided by the material manufacturer.

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Certificate Revision History: 02 September 2010 (This revision specifies the form of lactose as lactose monohydrate and adjusts the reference value and uncertainty in Table 5 to reflect the monohydrate content.); **09 July 2009** (Original certification date).

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) 926-4751; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.

APPENDIX A

Analysts at the laboratories listed below performed measurements that contributed to the value assignment of SRM 1849 Infant/Adult Nutritional Formula.

Abbott Nutrition; Columbus, OH, USA
Campbell Soup Company; Camden, NJ, USA[(a)]
Covance, Inc.; Madison, WI, USA[(a)]
Eurofins Danmark A/S; Kolding, Denmark
Eurofins Laboratories Ltd.; Wolverhampton, UK
Food and Drug Administration; Atlanta, GA, USA
Fonterra; Palmerston North, NZ
Fonterra; Waitoa, NZ
General Mills, Inc.; Minneapolis, MN, USA[(a)]
Hormel Foods Corporation; Austin, MN, USA[(a)]
Kraft; East Hanover, NJ, USA[(a)]
Kraft Foods; Glenview, IL, USA[(a)]
Krueger Food Laboratories; Cambridge, MA, USA[(a)]
Mead Johnson Nutritionals; Evansville, IN, USA
Nestlé USA; Dublin, OH, USA[(a)]
Novartis Nutrition Corporation; St. Louis Park, MN, USA[(a)]
PBM Nutritionals; Georgia, VT, USA

[(a)] This laboratory analyzed SRM 1849 as part of a GMA FIACC interlaboratory comparison exercise.

APPENDIX B

Methodological information reported by the collaborating laboratories (Appendix A) whose results were used for value assignment is summarized below. The number of laboratories using a particular method is provided in parentheses. The manufacturer's and NIST's methods are not included.

Proximates, Fatty Acids, Nitrogen, Amino Acids, Cholesterol, Calories, Lactose, and Nucleotides

Solids	Moisture determined by mass loss after oven-drying: Forced-air oven (6) Vacuum oven (8) Thermogravimetric analysis (1)
Ash	Mass loss after ignition in muffle furnace (14) Thermogravimetric analysis (2)
Fat	Acid digestion, ether extraction (6) Alkaline digestion, ether extraction (2) Mojonnier (4) Roese-Gottlieb (1) Summation of fatty acids by gas chromatography (GC) (6)
Fatty Acids	Gas chromatography (GC) (14)
Nitrogen	Kjeldahl (8) Thermal conductivity (3) Pyrolysis-GC (4)
Protein	Calculated; a factor of 6.38 was used to calculate protein from nitrogen results
Amino Acids	Hydrolysis – derivatization – liquid chromatography (4) Hydrolysis – ion exchange chromatography – ninhydrin (1)
Carbohydrates	Calculated; carbohydrate = solids - (protein + fat + ash)
Cholesterol	GC-flame ionization detector (3) GC/mass spectrometry (MS) (1)
Calories	Calculated; calories = 9(fat) + 4(protein) + 4(carbohydrate)
Lactose Monohydrate	Liquid chromatography – refractive index detection (7) Anion exchange chromatography – pulsed electrochemical detection (2)
Nucleotides	Liquid chromatography (LC) – absorbance detection (3)

Vitamins and Carotenoids

Retinol	Saponification – reversed phase LC (RPLC) – absorbance detection (4) Saponification – RPLC – fluorescence detection (1) Saponification – normal phase LC (NPLC) – absorbance detection (5) Extraction – LC – absorbance detection (1)
Cholecalciferol	Saponification – RPLC – absorbance detection (7) Saponification – NPLC – absorbance detection (2)
Tocopherols	Saponification – RPLC – fluorescence detection (6) Saponification – NPLC – fluorescence detection (2) Saponification – NPLC – absorbance detection (2)

Vitamin K	RPLC – reduction – fluorescence detection (6) Enzymatic reduction – fluorescence detection (2)
Ascorbic Acid	Absorbance (2) Fluorescence (6) LC – absorbance detection (3) Colorimetric titration (3)
Vitamin B_1 HCl	Microbiological (1) Digestion – fluorescence detection (1) Extration – ion pair chromatography (IPC) – fluorescence detection (4) Extraction – IPC – absorbance detection (2) Extraction – RPLC – fluorescence detection (2)
Vitamin B_2	Microbiological (1) Digestion – fluorescence detection (4) Extraction – RPLC – fluorescence detection (4) Extraction – IPC – absorbance detection (1)
Niacinamide	Microbiological (5) Extraction – IPC – absorbance detection (1) Extraction – RPLC – fluorescence detection (1) Extraction – LC – absorbance detection (2)
Pantothenic Acid	Microbiological (8) Extraction – LC – absorbance detection (2)
Vitamin B_6 HCl	Microbiological (4) Extraction – RPLC – fluorescence detection (5) Extraction – IPC – absorbance detection (1)
Vitamin B_{12}	Microbiological (7) Biosensor immunoassay (2)
Folic acid	Microbiological (7) RPLC – absorbance detection (1) Biosensor immunoassay (2)
Biotin	Microbiological (5) RPLC – fluorescence detection (1) Biosensor immunoassay (2)
Choline ion	Acid digestion - absorption spectrophotometry (4) Electrochemical detection (1) Acid digestion, electrochemical detection (1) Acid digestion, colorimetry (1) Enzymatic digestion, colorimetry (1)
Myo-inositol	Microbiological (2) GC (2)

Minerals and Trace Elements

Calcium	Inductively coupled plasma optical emission spectrometry (ICP-OES) (11) Flame atomic absorption spectrometry (FAAS) (1) Direct current plasma optical emission spectrometry (DCP) (1)
Chlorine	Electrochemical titration (2) Volhard (1)
Copper	ICP-OES (9)

	FAAS (1) DCP (1)
Chromium	ICP-OES (2) Graphite furnace atomic absorption spectrometry (GFAAS) (1)
Iodine	Ion-selective electrode (1) GC – electron capture detector (1) Inductively coupled plasma mass spectrometry (ICP-MS) (1) Colorimetry – arsenous reaction (1)
Iron	ICP-OES (10) FAAS (1) DCP (1)
Magnesium	ICP-OES (10) FAAS (1) DCP (1)
Manganese	ICP-OES (10) FAAS (1) DCP (1)
Molybdenum	ICP-OES (2) ICP-MS (1)
Phosphorus	ICP-OES (10) DCP (1) Colorimetry (1)
Potassium	ICP-OES (11) FAAS (1) DCP (1)
Selenium	ICP-OES (2) GFAAS (1) Hydride-generation atomic absorption spectrometry (1)
Sodium	ICP-OES (11) FAAS (1) DCP (1)
Zinc	ICP-OES (11) FAAS (1) DCP (1)