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

Date of Issue:
12 October 2023

Standard Reference Material® 1950
Metabolites in Frozen Human Plasma
CERTIFICATE OF ANALYSIS

Purpose: The certified values delivered by this Standard Reference Material (SRM) are intended for validating methods for determining metabolites such as fatty acids, electrolytes, vitamins, hormones, and amino acids in human plasma and similar materials. This SRM can also be used for comparison of measurement technologies used in metabolomic studies and for quality assurance when assigning values to in-house reference materials.

Description: A unit of SRM 1950 consists of five vials of one material. Each vial contains approximately 1 mL of frozen human plasma.

Certified Values: These values are traceable to International System of Units (SI) for amount of substance and mass, expressed in mass fraction (milligrams per kilogram or nanograms per gram), mass concentrations (milligrams per deciliter, micrograms per milliliter, nanograms per milliliter, or milligrams per liter) and amount concentration (millimoles per liter or micromoles per liter) [1].

Table 1. Certified Values for Cholesterol and Total Glycerides[a,b]

	Mass Concentration (mg/dL)	Amount-of-Substance Concentration (mmol/L)
Cholesterol	151.4 ± 3.3	3.917 ± 0.085
Total Glycerides (as triolein)	99.0 ± 2.1	1.12 ± 0.02

[a] Each certified value is the mean of NIST measurements using higher order reference methods.

[b] The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence; it expresses both the within method uncertainty and Type B components related to the analysis, consistent with the ISO/JCGM Guide [2]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the effects of the combined components of uncertainty, and k is a coverage factor corresponding to approximately 95 % confidence for each analyte. For the certified values shown, $k = 2$.

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

Additional Information: Additional information is provided in Appendix B.

Period of Validity: The certified values delivered by **SRM 1950** are valid within the measurement uncertainty specified until **30 September 2028**. 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).

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Table 2. Certified Values for Amino Acids[a,b]

	Mass Fraction (mg/kg)	Amount-of-substance Concentration (μmol/L)
Alanine	26.2 ± 2.2	300 ± 26
Glycine	18.0 ± 1.2	245 ± 16
Isoleucine	7.13 ± 0.42	55.5 ± 3.4
Leucine	12.90 ± 0.82	100.4 ± 6.3
Lysine	20.0 ± 1.9	140 ± 14
Methionine	3.26 ± 0.26	22.3 ± 1.8
Proline	19.9 ± 1.1	177 ± 9
Serine	9.87 ± 0.44	95.9 ± 4.3
Tyrosine	10.17 ± 0.53	57.3 ± 3.0
Valine	20.9 ± 1.2	182.2 ± 10.4

[a] Each certified value is the mean from the combination of the mean results from LC-MS/MS, GC-TOF-MS (MTBSTFA), and GC-TOF-MS (PCF), where available.

[b] The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence; it expresses both the observed difference between the results from the methods and their respective uncertainties, in addition to Type B components related to purity of the standards used, consistent with the ISO/JCGM Guide and its Supplement 1 [2–4]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the effects of the combined components of uncertainty, and k is a coverage factor corresponding to approximately 95 % confidence for each analyte. For the certified values, $k = 2$.

Table 3. Certified Values for Selected Vitamins and Carotenoids

	Mass Fraction (mg/kg)	Mass Concentration (μg/mL)
Retinol[a,b]	0.396 ± 0.034	0.404 ± 0.035
α-Tocopherol[a,b]	8.01 ± 0.22	8.18 ± 0.22
γ + β-Tocopherol[a,b]	1.67 ± 0.16	1.71 ± 0.17
Lutein[a,b]	0.067 ± 0.022	0.069 ± 0.023
Zeaxanthin[a,b]	0.021 ± 0.005	0.022 ± 0.005
β-Cryptoxanthin[a,b]	0.038 ± 0.003	0.039 ± 0.003
Total α–Carotene[a,b]	0.025 ± 0.005	0.026 ± 0.005
Total β-Carotene[a,b]	0.077 ± 0.004	0.079 ± 0.004
	Mass Fraction (ng/g)	**Mass Concentration (ng/mL)**
25-Hydroxyvitamin D_3[b,c]	24.27 ± 0.75	24.78 ± 0.77
5-Methyltetrahydrofolate[a,b]	12.11 ± 0.31	12.36 ± 0.32
Pyridoxal 5'-phosphate[a,b]	8.02 ± 0.45	8.19 ± 0.46

[a] The certified values are the means of the method means from measurements performed by NIST and CDC.

[b] The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence; it expresses both the observed difference between the results from the methods and their respective uncertainties, in addition to Type B components, consistent with the ISO/JCGM Guide and its Supplement 1 [2–4]. For carotenoids, the Type B components included sample preparation and purity of the standards; for vitamins, the Type B components included were related to the analysis. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the effects of the combined components of uncertainty, and k is a coverage factor corresponding to approximately 95 % confidence for each analyte. For the certified values, $k = 2$.

[c] The certified value for 25-hydroxyvitamin D_3 is the mean of the NIST LC-MS and NIST LC-MS/MS method means.

Table 4. Certified Values for Clinical Markers and Hormones[a,b]

	Mass Concentration (mg/dL)	Amount-of-substance Concentration (mmol/L)
Creatinine	0.6789 ± 0.0108	0.0600 ± 0.0009
Glucose	82.16 ± 1.00	4.560 ± 0.056
Urea	23.45 ± 0.49	3.90 ± 0.08
Uric Acid	4.274 ± 0.089	0.254 ± 0.005
	Mass Concentration (mg/L)	**Amount-of-substance Concentration (µmol/L)**
Homocysteine	1.150 ± 0.026	8.50 ± 0.20
	Mass Fraction (ng/g)	**Mass Concentration (ng/mL)**
Progesterone	1.452 ± 0.037	1.482 ± 0.038
Testosterone	2.169 ± 0.046	2.214 ± 0.047

[a] Each certified value is the mean of NIST measurements using higher-order reference methods.

[b] The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence; it expresses both the within-method uncertainty and Type B components related to the analysis, consistent with the ISO/JCGM Guide [2]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the effects of the combined components of uncertainty, and k is a coverage factor corresponding to approximately 95 % confidence for each analyte. For the certified values, $k = 2$.

Table 5. Certified Values for Electrolytes[a,b]

	Amount-of-substance Concentration (mmol/L)	Coverage Factor, k
Calcium	1.936 ± 0.024	2.19
Magnesium	0.696 ± 0.004	1.98
Potassium	3.665 ± 0.025	2.064
Sodium	141.76 ± 0.31	1.972

[a] Each certified value is the mean of NIST measurements using higher-order reference methods.

[b] The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence; it expresses both the within-method uncertainty and Type B components related to the analysis, consistent with the ISO/JCGM Guide [2]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the effects of the combined components of uncertainty, and k is a coverage factor corresponding to approximately 95 % confidence for each analyte.

Safety: SRM 1950 IS INTENDED FOR RESEARCH USE. This is a human-source material. SRM 1950 is a Biosafety Level 2 material and should be handled according to applicable federal, state, and/or local regulations and according to policies and procedures of recipient's organization. The supplier has reported that each donor unit of plasma used in the preparation of this product was tested by FDA-licensed tests and found to be negative for human immunodeficiency virus (HIV), HIV-1 antigen, hepatitis B, surface antigen, and hepatitis C [5].

Storage: The original unopened vials of SRM 1950 should be stored at –60 °C or lower.

Use: SRM 1950 is provided as frozen plasma that should be allowed to thaw at room temperature for at least 30 min under subdued light. After the material is thawed, it should be used immediately. The contents of the vial should then be gently mixed prior to removal of a test portion for analysis. Precautions should be taken to avoid exposure to strong UV light and direct sunlight.

Value Assignment: Means of data sets from individual methods were combined to provide assigned values. The measured plasma density is 1.020 86 g/mL with a standard deviation of 0.000 20 g/mL; this uncertainty was incorporated in values that are reported relative to units of volume.

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Certificate Revision History: 12 October 2023 (Certified fatty acid values changed to non-certified values; certified value for histidine and non-certified value for cystine removed; certified value threonine value changed to non-certified value; certified cortisol value changed to non-certified value; change of expiration date; updated format; editorial changes); **16 December 2020** (Editorial changes); **19 June 2020** (Reference value for zinc removed from Table A6; editorial changes); **23 September 2019** (Reference value for vitamin D-binding protein added to Table A6; editorial changes); **21 April 2016** (Removed reference values for glutamic acid, ornithine, retinyl palmitate, and retinyl stearate; change of expiration date; editorial changes); **26 January 2016** (Editorial changes); **07 November 2012** (Corrected conversion from µg/g to µmol/L for several fatty acids in Tables 2 and 3; editorial changes); **31 August 2011** (Update of homocysteine units); **14 July 2011** (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.

* * * * * * * End of Certificate of Analysis * * * * * * *

APPENDIX A

Non-Certified Values: Non-certified values are suitable for use in method development, method harmonization, and process control but do not provide metrological traceability to the International System of Units (SI) or other higher-order reference system [1]. Values are metrologically traceable to the measurement procedures as indicated in the footnotes.

Table A1. Non-Certified Values for Fatty Acids

Lipid Name	Chemical Name (Common Name)	Mass Fraction (µg/g)	Amount-of-Substance Concentration (µmol/L)
C12:0	Dodecanoic Acid (Lauric Acid) [a,b]	1.86 ± 0.11	9.47 ± 0.57
C16:0	Hexadecanoic Acid (Palmitic Acid) [a,b]	594 ± 19	2364 ± 77
C16:1 n-7	(Z)-9-Hexadecenoic Acid (Palmitoleic Acid) [a,b]	53.5 ± 6.4	215 ± 26
C18:0	Octadecanoic Acid (Stearic Acid) [a,b]	179 ± 12	644 ± 41
C18:3 n-3	(Z,Z,Z)-9,12,15-Octadecatrienoic Acid (α-Linolenic Acid) [a,b]	14.9 ± 1.0	54.6 ± 3.6
C18:1 n-9	(Z)-9-Octadecenoic Acid (Oleic Acid) [a,b]	447 ± 43	1614 ±154
C22:0	Docosanoic Acid (Behenic Acid) [a,b]	15.9 ± 1.5	47.8 ± 4.6
C14:0	Tetradecanoic Acid (Myristic Acid) [a,b]	17.9 ± 3.8	80.1 ± 17.0
C14:1	(Z)-9-Tetradecenoic Acid (Myristoleic Acid)[c,d]	1.57 ± 0.03	7.1 ± 0.1
C15:0	Pentadecanoic Acid[b,e]	1.08 ± 0.01	4.56 ± 0.04
C17:0	Heptadecanoic Acid (Margaric Acid)[d,e]	4.7 ± 0.2	17.6 ± 0.7
C18:3 n-6	(Z,Z,Z)-6,9,12-Octadecatrienoic Acid (γ-Linolenic Acid)[a,b]	10.9 ± 2.3	39.9 ± 8.5
C18:2 n-6	(Z,Z)-9,12-Octadecadienoic Acid (Linoleic Acid) [a,b,f]	780 ± 39	2838 ±143
C18:1 n-7	(Z)-11-Octadecenoic Acid (Vaccenic Acid)[c,d]	37.7 ± 0.9	136 ± 3
C20:0	Eicosanoic Acid (Arachidic Acid)[c,d]	5.5 ± 0.2	18.0 ± 0.5
C20:1	(Z)-11-Eicosenoic Acid (Gondolic Acid)[c,d]	3.5 ± 0.1	11.5 ± 0.5
C20:2	(Z,Z)-1,14-Eicosadienoic Acid[c,d]	5.7 ± 0.2	18.8 ± 0.6
C20:3 n-6	(Z,Z,Z)-8,11,14-Eicosatrienoic Acid (Dihomo-γ-Linolenic Acid)[c,d]	41.8 ± 1.1	139 ± 4
C20:4 n-6	(Z,Z,Z,Z)-5,8,11,14-Eicosatetraenoic Acid (Arachidonic Acid)[a,b]	293 ± 54	984 ±180
C20:5 n-3	(Z,Z,Z,Z,Z) -5,8,11,14,17-Eicosapentaenoic Acid (EPA)[c,d]	11.4 ± 0.1	38.6 ± 0.5
C22:1	(Z)-13-Docosenoic Acid (Erucic Acid)[c,d]	1.1 ± 0.4	3.4 ± 1.3
C22:4 n-6	(Z,Z,Z,Z)-7,10,13,16-Docosatetraenoic Acid[c,d]	8.3 ± 0.2	25.5 ± 0.6
C22:5 n-3	(Z,Z,Z,Z,Z)-7,10,13,16,19-Docosapentaenoic Acid (DPA)[c,d]	12.5 ± 0.2	38.5 ± 0.7
C22:5 n-6	(Z,Z,Z,Z,Z)-4,7,10,13,16-Docosapentaenoic Acid[c,d]	6.3 ± 0.1	19.5 ± 0.4
C22:6 n-3	(Z,Z,Z,Z,Z,Z)-4,7,10,13,16,19-Docosahexaenoic Acid (DHA)[a,b]	37.9 ± 6.8	118 ± 21
C24:0	Tetracosanoic Acid (Lignoceric Acid)[c,d]	16.8 ± 0.9	46.6 ± 2.6
C24:1	(Z)-15-Tetracosenoic Acid (Nervonic Acid)[c,d]	25.6 ± 1.2	71.3 ± 3.2

[a] Values are weighted means of the results from analyses at NIST using GC-MS and GC-FID and from CDC using GC-MS [6–10].

[b] The expanded uncertainty about the mean is calculated by combining a pooled within-method variance with a between-method variance and a coverage factor $k = 2$ (approximately 95 % confidence) following the ISO/JCGM Guides [2,4,8–10].

[c] Values are from analyses at the CDC using GC-MS.

[d] The expanded uncertainty U is calculated as $U = ku_c$, where u_c is one standard deviation of the analyte mean and k is determined from the Student's t-distribution corresponding to the associated degrees of freedom and 95 % confidence level for each analyte. For the values shown, $k = 2$.

[e] Values which are from NIST GC-FID analyses.

[f] The value for linoleic acid (C18:2 n-6) was based upon the NIST GC-FID and CDC GC-MS results.

Table A2. Non-Certified Values for Amino Acids

	Mass Fraction (mg/kg)	Amount-of-substance Concentration (μmol/L)
Arginine[a,b]	13.89 ± 0.40	81.4 ± 2.3
Cysteine[a,b]	5.26 ± 0.81	44.3 ± 6.9
Phenylalanine[a,b]	8.2 ± 1.1	51 ± 7
Threonine[b,c]	13.94 ± 0.70	119.5 ± 6.1

(a) Values are based on the LC-MS/MS method means.

(b) The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence; it expresses both the observed difference between the results from the methods and their respective uncertainties, in addition to Type B components related to purity of the standards used, consistent with the ISO/JCGM Guide and its Supplement 1 [2–4]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the effects of the combined components of uncertainty, and k is a coverage factor corresponding to approximately 95 % confidence for each analyte. For the values shown, $k = 2$.

(c) Value is the mean from the combination of the mean results from LC-MS/MS, GC-TOF-MS (MTBSTFA), and GC-TOF-MS (PCF).

Table A3. Non-Certified Values for Vitamins and Carotenoids

	Mass Fraction (mg/kg)	Mass Concentration (μg/mL)	Coverage Factor, k
Trans-Lycopene[a,b]	0.14 ± 0.01	0.14 ± 0.01	2.36
Total Lycopene[a,b]	0.32 ± 0.02	0.33 ± 0.02	2.35
Trans-β-Carotene[a,b]	0.071 ± 0.005	0.072 ± 0.005	2.38
Cis-β-Carotene[a,b]	0.0040 ± 0.0003	0.0041 ± 0.0003	2.39
	Mass Fraction (ng/g)	**Mass Concentration (ng/mL)**	**Coverage Factor, k**
25-Hydroxyvitamin D_2[b,c]	0.51 ± 0.17	0.52 ± 0.17	2
Folic acid[b,d]	1.48 ± 0.44	1.51 ± 0.45	2

(a) Values for carotenoids are based upon the mean of measurements performed by CDC.

(b) The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence; it expresses both the observed difference between the results from the methods and their respective uncertainties, in addition to Type B components, consistent with the ISO/JCGM Guide and its Supplement 1 [2–4]. For carotenoids, the Type B components included sample preparation and purity of the standards; for vitamins, the Type B components included were related to the analysis. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the effects of the combined components of uncertainty, and k is a coverage factor corresponding to approximately 95 % confidence for each analyte.

(c) Value for 25-hydroxyvitamin D_2 is the mean of the NIST LC-MS and NIST LC-MS/MS method means.

(d) Value for folic acid is the mean of the NIST and CDC method means.

Table A4. Values of Potential Interest to Users for Additional Vitamers

	Amount-of-substance Concentration (nmol/L)
Total folate[a,b]	30.6
4-Pyridoxic acid[a]	28.7

(a) Values are the means of results provided by the CDC. These values are provided without uncertainties because insufficient information is available to assess them.

(b) Microbiological analysis.

Table A5. Non-Certified Value for Cortisol[a,b]

	Mass Fraction (ng/g)	Mass Concentration (ng/mL)
Cortisol	82.2 ± 1.7	83.9 ± 1.7

[a] Value is the mean of NIST measurements using a higher-order reference method.

[b] The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence; it expresses both the within-method uncertainty and Type B components related to the analysis, consistent with the ISO/JCGM Guide [2]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the effects of the combined components of uncertainty, and k is a coverage factor corresponding to approximately 95 % confidence for each analyte. For the value shown, $k = 2$.

Table A6. Non-Certified Values for Trace Elements, Selenium Species, Total Protein, Bilirubin, and Vitamin D-Binding Protein

	Mass Fraction (mg/kg)	Coverage Factor, k
Copper[a,b]	1.008 ± 0.008	2.09
Selenium[a,b]	0.1055 ± 0.0038	2.2

	Mass Fraction (µg/kg)	Coverage Factor, k
Selenoprotein P[a,b]	50.2 ± 4.3	2.6
Glutathione Peroxidase[a,b]	23.6 ± 1.3	2.5
Seleno-Albumin[a,b]	28.2 ± 2.6	2.6

	Mass Concentration	Coverage Factor, k
Total Protein (g/L)[a,b]	59.1 ± 1.7	2
Bilirubin (mg/dL)[a,b]	0.344 ± 0.023	2.11
Vitamin D-Binding Protein (mg/L)[b,c,d]	175 ± 18	2.0

[a] Each value is the mean of NIST measurements using a single method.

[b] The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence; it expresses both the within-method uncertainty and Type B components related to the analysis, consistent with the ISO/JCGM Guide [2]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the effects of the combined components of uncertainty, and k is a coverage factor corresponding to approximately 95 % confidence for each analyte.

[c] The uncertainty for vitamin D-binding protein also incorporates the observed difference between the results from the methods [2–4].

[d] Value is the mean of means of measurements from NIST and University of Washington.

Table A7. Non-Certified Values for PFCs[a,b]

	Mass Fraction (ng/g)	Mass Concentration (ng/mL)
Perfluorooctanoic Acid (PFOA)	3.21 ± 0.06	3.27 ± 0.06
Perfluorononanoic Acid (PFNA)	0.705 ± 0.028	0.720 ± 0.028
Perfluorodecanoic Acid (PFDA)	0.315 ± 0.006	0.322 ± 0.007
Perfluoroundecanoic Acid (PFUnA)	0.182 ± 0.003	0.186 ± 0.003
Perfluorohexansulfonate (PFHxS)	3.19 ± 0.08	3.25 ± 0.08
Perfluorooctanesulfonic Acid (PFOS)	10.43 ± 0.12	10.64 ± 0.13

[a] Values are weighted means of the results from analyses at NIST using LC-MS/MS with two different sample preparation schemes and different chromatographic separations [8,9]. The concentrations reflect the total of both branched and linear forms of the analytes.

[b] The uncertainty provided with each value is an expanded uncertainty about the mean [10], with coverage factor $k = 2$ (approximately 95 % confidence), calculated by combining a pooled within-method variance with a between-method variance following the ISO/JCGM Guides [2,3,10].

Determination of Fatty Acids (NIST): Mass fractions of fatty acids in SRM 1950 were determined using two different extraction procedures and two analytical methods. An internal standard solution containing stearic-d_{35} acid and myristic-d_{27} acid was used. One set of samples was saponified in methanolic KOH and esterified using sulfuric acid in methanol. A second set was treated with sodium methoxide in methanol followed by boron trifluoride [6]. Both sets of samples were analyzed by GC with flame ionization detection (FID) and GC-MS. 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.

Determination of Fatty Acids (CDC): Total fatty acids were determined using ID-GC-MS based on Langerstedt's method [7]. This procedure employs hydrolysis of fatty acids from cholesteryl esters, triglycerides, and phospholipids using sequential addition of acetonitrile:hydrochloric acid and methanol:sodium hydroxide in the presence of heat. Total fatty acids were extracted in hexane, concentrated, derivatized using pentafluorobenzyl bromide (PFB), and reconstituted in hexane. Twenty-four fatty acids were quantified using 10 isotopically labeled internal standards. Fragmentation of fatty acid-PFB esters by negative chemical ionization resulted in a reproducible loss of the PFB moiety giving a stable carboxylate anion on a capillary column (30 m × 0.25 mm × 0.25 μm) with helium as the carrier gas.

Analytical Approach for Determination of Selenium Species: The selenium species selenoprotein P, glutathione peroxidase, and seleno-albumin were determined by ID-ICP-MS. Separation of selenoproteins was performed by affinity chromatography on heparin-sepharose and blue-sepharose stationary phases that had been packed into PEEK columns (4 mm × 50 mm). Proteins were eluted with 1.5 mol/L ammonium acetate. Quantitation was based upon ID with ^{77}Se.

Analytical Approach for Determination of Total Protein: Total protein mass concentration was determined using a biuret method [11]. Spectrophotometric measurements were calibrated using SRM 927d Bovine Serum Albumin (7 % Solution).

Analytical Approach for Determination of Bilirubin: The mass concentration of bilirubin was determined using a spectrophotometric reference method developed by Doumas [12].

Analytical Approach for Determination of Vitamin D-Binding Protein: Vitamin D-binding protein (VDBP) was measured by NIST and the University of Washington (UW) with the LC-MS/MS methods as described below.

Determination of VDBP by NIST*:* Seven vials of SRM 1950 were randomly selected and analyzed by ID-LC-MS/MS in triplicate on different days. Samples were prepared gravimetrically using trifluoroethanol to denature the proteins as previously described [13]. Following tryptic digestion for 19 hours, peptides containing one Leucine residue with $^{13}C_6$ and ^{15}N were added to each sample. The two transitions with the greatest intensity for peptides TSALSAK and VLEPTLK were monitored during ID-LC-MS/MS. The total protein concentration was calculated as the mean of the two peptides. Concentrations were calculated in units milligrams per liter using the mean of the range of molecular masses of the common isoforms with or without glycosylation (51 200 g/mol and 51 900 g/mol).

Determination of VDBP by University of Washington (UW)*:* Seven vials of SRM 1950 were randomly selected and sent to UW for analysis by ID-LC-MS/MS. The digestion procedure used at UW was similar to the one used at NIST [14]. However, peptides ELPEHTVK and VLEPTLK were used during LC-ID-MS/MS. Also, the labeled peptide standards were added to the samples at the beginning of the sample processing and the total digestion time was 30 minutes.

Analytical Approach for Determination of Perfluorinated Compounds (PFCs): Perfluorinated compounds were determined by LC-MS/MS in the negative-ion mode with ^{13}C-labeled PFCs as internal standards. In the first method, samples were spiked with the internal standards and mixed with 50 % formic acid in water (volume fractions). The PFCs were isolated by SPE with weak anion exchange cartridges. Chromatographic separation was achieved on either a C_8 or a pentafluorophenyl column with gradient elution, and the mobile phases were comprised of methanol and 20 mmol/L ammonium acetate. In the second method, samples were spiked with the internal standards and plasma proteins were precipitated with acetonitrile. After centrifugation and a solvent exchange to methanol, PFCs were isolated from the supernatants by SPE with graphitized carbon cartridges. Chromatographic separation was achieved on either a C_8 or a pentafluorophenyl column with gradient elution, and the mobile phases were comprised of methanol and 20 mmol/L ammonium acetate.

Analytical Approach for Determination of Amino Acids: Amino acids were quantified using ID liquid chromatography (LC) tandem MS (ID-LC-MS/MS), GC coupled to time-of-flight (TOF) MS (GC-TOF-MS), with two different types of derivatization, and two-dimensional GC×GC-TOF-MS, as described in reference 15. Cysteine thiols have been shown to oxidize in plasma forming cystine dimers or to form other disulfide bonds such as those of glutathione [16–18]. No specific reducing agents were added to prevent thiol reactions in this study; however, the experimental approach was optimized to minimize potential thiol reactions by keeping plasma samples at or below 4 °C at all times during LC analyses or until the derivatization step was reached during GC-MS analyses.

For the LC-MS/MS, GC-MS, and GC×GC-TOF-MS methods, amino acids were enriched from plasma using standard methanol precipitation and spiked with isotopically labeled amino acid analogs. For LC-MS/MS, analytes were separated on a mixed mode (ion-exclusion and reversed-phase) analytical column, and multiple reaction monitoring (MRM) was performed in a triple quadrupole mass spectrometer for two distinct fragmentation transitions from each ion. For GC-TOF-MS and GC×GC-TOF-MS, the methanol layer was evaporated to dryness and derivatized with N-(*t*-butyldimethylsilyl)N-methyltrifluoroacetamide (MTBSTFA). The derivatized sample was injected into the GC and separated on two columns in series: a 5 % diphenyl/95 % dimethyl polysiloxane (mole fraction) column and a 50 % diphenyl/50 % dimethyl polysiloxane (mole fraction) column. GC×GC-TOF-MS was a two-dimensional separation with cryotrapping between the two columns and a modulation period of 3 s. GC-TOF-MS analysis was performed in one dimension without cryotrapping.

For the second GC-TOF-MS method, plasma was spiked with isotopically labeled amino acid analogs. Derivatization was performed in the plasma matrix with propyl chloroformate (PCF)/propanol. Derivatized products were extracted using chloroform for injection into the GC and separated on two 5 % diphenyl/95 % dimethyl polysiloxane (mole fraction) columns in series, in one dimension without cryotrapping between the columns.

Analytical Approach for Determination of Carotenoids (NIST*)*: Retinol and carotenoids were measured at NIST by using combinations of two LC methods with absorbance detection: (1) a polymeric C_{18} column [19] with UV/visible absorbance detection [20,21] and (2) a C_{18} column with different selectivity and absorbance detection of retinol and carotenoids and fluorescence detection of tocopherols [21,22]. Proteins in the plasma were precipitated with ethanol containing an internal standard. Analytes were extracted into hexane, which was evaporated. The reconstituted extracts were then analyzed by LC with absorbance and/or fluorescence detection.

Analytical Approach for Determination of Carotenoids (CDC): Fat-soluble micronutrients were measured at CDC using reversed-phase LC with photodiode array detection [23]. Plasma was mixed with an ethanol solution containing two internal standards, retinyl butyrate and β-apo-8′-carotenal. Micronutrients were extracted from the aqueous phase into hexane and dried under vacuum. The extract was redissolved in ethanol and acetonitrile and filtered to remove any insoluble material. An aliquot of the filtrate was injected onto a high carbon load C_{18} column (150 mm × 4.6 mm × 3 µm particle size) and eluted with a gradient consisting of ethanol and acetonitrile. Absorbance was monitored at 450 nm for carotenoids.

Analytical Approach for Determination 25(OH)D_2 by ID-LC-MS: Plasma and an internal standard solution containing $^2H_3$-25(OH)D_2 were combined in glass tubes, proteins were precipitated, and the metabolites were extracted into hexane twice. The hexane phases were combined and evaporated to dryness at 40 °C under nitrogen. The residues were reconstituted and were further clarified using centrifuge filters. Extracts were analyzed by LCMS using: (-1) a deactivated C_{18} stationary phase and (2) a cyanopropyl stationary phase. Atmospheric pressure

chemical ionization (APCI) MS detection with positive polarity was used for both chromatographic methods. The $[M - H_2O + H]^+$ ions were monitored for all species and were used for quantitation. The ions monitored included *m/z* 395 for 25(OH)D_2 and *m/z* 398 for $^2H_3$-25(OH)D_2.

Analytical Approach for Determination of 25(OH)D_2 by ID-LC-MS/MS: Two grams to 2.5 g of plasma were combined with water (to avoid protein precipitation when samples were spiked with internal standard solutions), and an internal standard solution containing $^2H_3$-25(OH)D_2. After equilibration at room temperature for 1 h, the pH of each sample was adjusted to pH 9.8 ± 0.2 with carbonate buffer. Analytes were extracted from the plasma matrix with a mixture of hexane and ethyl acetate. The combined extracts were dried under nitrogen at 45 °C, and the residues were reconstituted with methanol for LC-MS/MS analysis. APCI in the positive-ion mode and MRM mode were used. The transitions at *m/z* 413 → *m/z* 395 for 25(OH)D_2 and *m/z* 416 → *m/z* 398 for $^2H_3$-25(OH)D_2 were monitored [24].

Analytical Approach for Determination of Folic Acid (NIST): Folic acid was measured using a JCTLM-approved ID-LC-MS/MS method using $^{13}C_5$-folic acid as internal standard [25]. 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. For folic acid, the transitions at *m/z* 442 → *m/z* 295 (unlabeled) and *m/z* 447 → *m/z* 295 (labeled) were monitored.

Analytical Approach for Determination of Folic Acid (CDC): The folate vitamer folic acid were measured by ID-LC-MS/MS using $^{13}C_5$-folic acid as internal standard [26,27]. This is a JCTLM-approved method. Folate species were isolated from plasma by solid-phase extraction (SPE) with phenyl cartridges. The folate vitamers were separated on a C_8 column under isocratic conditions with an organic mobile phase containing acetic acid. The transitions monitored for folic acid were the same as those listed above for the NIST determination.

Analytical Approach for Determination of Folate (CDC): Folate was also measured by a microbiological assay using a 96 -well plate microtiter method [28,29]. Diluted plasma was added to an assay medium inoculated with *Lactobacillus casei* (NCIB 10463) and containing all of the nutrients except folic acid necessary for growth of *L. casei*. The microtiter plate was incubated for 42 h at 37 °C. Because the growth of *L. casei* is proportional to the amount of total folate present in the plasma sample, the folate concentration was quantified by measuring the turbidity of the inoculated assay medium at 590 nm in a micro plate reader. The assay was calibrated with 5-methyltetrahydrofolate.

Analytical Approach for Determination of Vitamin B_6 (CDC): The vitamin B_6 vitamer 4-pyridoxic acid was determined by LC with chlorite post column-derivatization and fluorescence detection after protein precipitation with metaphosphoric acid and sample filtration [30,31]. The B_6 vitamer was separated under isocratic conditions on a C_{18} column with a mobile phase comprised of aqueous phosphate buffer (with 0.2 % acetonitrile) and methanol. The initial mobile phase was comprised of 100 % aqueous buffer, and a linear gradient from 0 % to 30 % methanol was employed after elution of the B_6 vitamer to facilitate column cleanup between injections.

Analytical Approach for Determination of Cortisol: Value assignment of the cortisol mass fraction was based on the NIST reference method (JCTLM-approved) for cortisol [32], which involves spiking the plasma with cortisol-d_3, acidifying the sample, putting the sample through an SPE C_{18} cartridge, liquid-liquid extraction, and analysis by LC-MS/MS using a C_{18} column and monitoring two transitions each for the unlabeled forms: *m/z* 363 → *m/z* 327 and *m/z* 363 → *m/z* 121 and labeled forms: *m/z* 366 → *m/z* 330 and *m/z* 366 → *m/z* 121.

Analytical Approach for Determination of Elements: Copper and selenium were determined using a single method [33]. The mass fraction for selenium is based upon measurements using ID-ICP-MS. The mass fraction for copper is based on measurements using ICP-MS with standard additions.

Analytical Approach for Determination of Cholesterol and Total Glycerides: The cholesterol mass fraction was determined using the NIST isotope dilution (ID) gas chromatography (GC) mass spectrometry (MS) (ID-GC-MS) definitive method [34,35]. This method is an approved higher-order reference measurement procedure according to the Joint Committee for Traceability in Laboratory Medicine (JCTLM) [36]. This procedure employs hydrolysis of cholesterol esters using potassium hydroxide (KOH) in ethanol, followed by extraction with hexane, and derivatization of cholesterol using *bis*(trimethylsilyl)acetamide. Cholesterol-25,26,27-$^{13}C_3$ was used as the internal standard.

Value assignment of the mass concentration of total glycerides (as triolein) was based upon the NIST ID-GC-MS definitive method [37]. This method is recognized as a higher-order reference measurement procedure by the JCTLM. The method involves hydrolysis of triglycerides with ethanolic KOH, deionization, reaction with

butylboronic acid in pyridine, and derivatization with N-methyl-N-trimethylsilyltrifluoroacetamide. Tripalmitin-1,2,3-$^{13}C_3$ was used as the internal standard.

Analytical Approach for Determination of Fat-Soluble Vitamins and Carotenoids: Value assignment of the mass fractions of the vitamins and carotenoids in SRM 1950 was based on the combination of results provided from several different analytical methods at NIST and CDC. NIST provided measurements by using a combination of different LC methods with different detection modes as described below.

Determination of Vitamins A and E and Carotenoids (NIST): Retinol and carotenoids were measured at NIST by using combinations of two LC methods with absorbance detection: (1) a polymeric C_{18} column [19] with UV/visible absorbance detection [20,21] and (2) a C_{18} column with different selectivity and absorbance detection of retinol and carotenoids and fluorescence detection of tocopherols [21,22]. Proteins in the plasma were precipitated with ethanol containing an internal standard. Analytes were extracted into hexane, which was evaporated. The reconstituted extracts were then analyzed by LC with absorbance and/or fluorescence detection.

Determination of Vitamins A and E and Carotenoids (CDC): Fat-soluble micronutrients were measured at CDC using reversed-phase LC with photodiode array detection [23]. Plasma was mixed with an ethanol solution containing two internal standards, retinyl butyrate and β-apo8′-carotenal. Micronutrients were extracted from the aqueous phase into hexane and dried under vacuum. The extract was redissolved in ethanol and acetonitrile and filtered to remove any insoluble material. An aliquot of the filtrate was injected onto a high carbon load C_{18} column (150 mm × 4.6 mm × 3 µm particle size) and eluted with a gradient consisting of ethanol and acetonitrile. Absorbance was monitored at 300 nm for vitamin E, 325 nm for vitamin A, and 450 nm for carotenoids.

Analytical Approach for Determination of Vitamin D Metabolites: The vitamin D metabolite, 25-hydroxyvitamin D_3, [25(OH)D_3], was measured at NIST using ID-LCMS and ID-LC-MS/MS [24], with two different types of chromatographic separations.

Determination of 25(OH)D_3 by ID-LC-MS: Plasma and an internal standard solution containing $^2H_6$-25(OH)D_3 were combined in glass tubes, proteins were precipitated, and the metabolites were extracted into hexane twice. The hexane phases were combined and evaporated to dryness at 40 °C under nitrogen. The residues were reconstituted and were further clarified using centrifuge filters. Extracts were analyzed by LC-MS using: (1) a deactivated C_{18} stationary phase and (2) a cyanopropyl stationary phase. Atmospheric pressure chemical ionization (APCI) MS detection with positive polarity was used for both chromatographic methods. The $[M - H_2O + H]^+$ ions were monitored for all species and were used for quantitation. The ions monitored included *m/z* 383 for 25(OH)D_3 and *m/z* 389 for $^2H_6$-25(OH)D_3.

Determination of 25(OH)D_3 by ID-LC-MS/MS: Two grams to 2.5 g of plasma were combined with water (to avoid protein precipitation when samples were spiked with internal standard solutions), and an internal standard solution containing $^2H_3$-25(OH)D_3. After equilibration at room temperature for 1 h, the pH of each sample was adjusted to pH 9.8 ± 0.2 with carbonate buffer. Analytes were extracted from the plasma matrix with a mixture of hexane and ethyl acetate. The combined extracts were dried under nitrogen at 45 °C, and the residues were reconstituted with methanol for LC-MS/MS analysis. APCI in the positive-ion mode and MRM mode were used. The transitions at *m/z* 401 → *m/z* 383 for 25(OH)D_3 and *m/z* 404 → *m/z* 386 for $^2H_3$-25(OH)D_3 were monitored.

Analytical Approach for Determination of Water-Soluble Vitamins: Water-soluble vitamins were measured by NIST and CDC using combinations of LC methods with fluorescence or tandem mass spectrometric detection as described below.

Determination 5-Methyltetrahydrofolate (NIST): 5-methyltetrahydrofolate were measured in SRM 1950 using a JCTLM-approved ID-LC-MS/MS method using $^{13}C_5$-5-methyltetrahydrofolate as internal standard [25]. A gradient LC method with a water/methanol/formic acid mobile phase and a pentafluorophenyl column were used for the positive ion mode LCMS/MS determination. For 5-methyltetrahydrofolate, the transitions at *m/z* 460 → *m/z* 313 (unlabeled) and *m/z* 465 → *m/z* 313 (labeled) were monitored.

Determination of 5-Methyltetrahydrofolate (CDC): The folate vitamer 5-methyltetrahydrofolate was measured by ID-LC-MS/MS using $^{13}C_5$-5-methyltetrahydrofolate as internal standard [26,27]. This is a JCTLM-approved method. Folate species were isolated from plasma by solid-phase extraction (SPE) with phenyl cartridges. The folate vitamers were separated on a C_8 column under isocratic conditions with an organic mobile phase containing acetic acid. The transitions monitored for 5-methyltetrahydrofolate were the same as those listed above for the NIST determination.

Determination of Vitamin B_6 (NIST): Vitamin B_6 was determined as pyridoxal 5'-phosphate by ID-LC-MS/MS. A labeled internal standard (pyridoxal-[$^2H_3$]-5'-phosphate) was added to the plasma and allowed to equilibrate for 30 min. Plasma proteins were precipitated by the addition of aqueous trichloroacetic acid followed by incubation at room temperature. After centrifugation, supernatants were analyzed by LC-MS/MS. The transitions at *m/z* 248 → *m/z* 150 (unlabeled) and *m/z* 251 → *m/z* 153 (labeled) were monitored.

Determination of Vitamin B_6 (CDC): The vitamin B_6 vitamer pyridoxal 5'-phosphate was determined by LC with chlorite post-column derivatization and fluorescence detection after protein precipitation with metaphosphoric acid and sample filtration [30,31]. The B_6 vitamer was separated under isocratic conditions on a C_{18} column with a mobile phase comprised of aqueous phosphate buffer (with 0.2 % acetonitrile) and methanol. The initial mobile phase was comprised of 100 % aqueous buffer, and a linear gradient from 0 % to 30 % methanol was employed after elution of the B_6 vitamer to facilitate column cleanup between injections.

Analytical Approach for Determination of Glucose: Value assignment of the glucose mass fraction was based on a modification of the NIST reference method for glucose, which involves ID-GC-MS and conversion of glucose into a dibutylboronate acetate derivative [38,39]. This method is an approved higher-order reference measurement procedure according to the JCTLM [36]. For certification of SRM 1950 this procedure was modified in that the plasma was not passed through an ion-exchange resin prior to concentration, freeze-drying, and derivatization.

Analytical Approach for Determination of Creatinine: Creatinine was determined using an ID-LC-MS method [40] that is similar to a method developed at the Laboratory of the Government Chemist [41] and is approved by the JCTLM as a higher-order reference measurement procedure.

Analytical Approach for Determination of Urea and Uric Acid: Urea was determined using a modification of the ID-GC-MS method described in reference 42, approved by JCTLM, in which the plasma was spiked with urea-^{18}O, passed through an SPE cartridge, concentrated, then derivatized to 6-methyluracil overnight. Uric acid was determined using a modification of the ID-GC-MS method described in reference 43, approved by the JCTLM, in which plasma samples were spiked with uric acid-$^{15}N_2$, mixed with 0.001 mol/L ammonium hydroxide, passed through a strong anion exchange resin, eluted from the column with 1 mol/L acetic acid, freeze-dried, and derivatized with MTBSTFA.

Analytical Approach for Determination of Homocysteine: Homocysteine was determined using an ID-GC-MS method similar to a method developed at the University of Colorado Health Sciences Center [44,45]. Plasma was spiked with homocystine-d_8, hydrolyzed with dithiothreitol in sodium hydroxide solution to break disulfide bonds and release homocysteine and homocysteine-d_4, which were isolated on an anion exchange resin, concentrated, derivatized, and analyzed by GC-MS with selective ion monitoring at *m/z* 420 and *m/z* 424 with confirmation made by monitoring *m/z* 318 and *m/z* 322 [46]. This method is approved by the JCTLM as a higher-order reference measurement procedure.

Analytical Approach for Determination of Hormones: The value assignments for the progesterone and testosterone mass fractions were based upon the LC-MS/MS analytical approaches described below.

Determination of Progesterone: Value assignment of the progesterone mass fraction was based on the NIST reference method (JCTLM-approved), for progesterone [47,48], which involves spiking the plasma with progesterone-$^{13}C_2$, a liquid-liquid extraction, and analysis using LC-MS/MS with a C_{18} column and monitoring the transitions for the unlabeled form, *m/z* 315 → *m/z* 97 and the labeled form, *m/z* 317 → *m/z* 99.

Determination of Testosterone: Value assignment of the testosterone mass fraction was based on the NIST reference method (JCTLM-approved) for testosterone [49], which involves spiking the plasma with testosterone-d_3, SPE, and analysis using LC-MS/MS with a C_{18} column and monitoring the transitions for the unlabeled form, *m/z* 289 → *m/z* 97, and the labeled form, *m/z* 292 → *m/z* 97.

Analytical Approach for Determination of Elements: Calcium, magnesium, potassium, and sodium were determined using a single method [33]. The certified mass fractions for calcium, magnesium, and potassium are based on measurements using isotope dilution collision cell technology (CCT) inductively coupled plasma mass spectrometry, (ID-CCT-ICP-MS) [50–52], which is a method approved by the JCTLM. The certified mass fraction for sodium is based on measurements using NIST's gravimetric definitive method [52,53], a JCTLM-approved method, without ion exchange.

Period of Validity: The non-certified values delivered by **SRM 1950** are valid within the measurement uncertainty specified until **30 September 2028**. The non-certified values 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 Certificate of Analysis 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

Source and Preparation: SRM 1950 is designed to represent "normal" human plasma. Plasma was obtained from 100 individuals who had undergone an overnight fast prior to blood draw by Bioreclamation (Hicksville, NY). Lithium heparin was used as the anticoagulant. A rapid glucose oxidase test was used to exclude individuals who did not adhere to the fasting requirement. Plasma from an equal number of men and women in a narrow adult age range (40 to 50 years) was used. Racial distribution of the donors reflected the distribution in the U.S. population, i.e., approximately 77 % white, 12 % African American or black, 2 % American Indian or Alaskan Native, 4 % Asian, 5 % "other," with approximately 15 % of the total taken from individuals of Hispanic origin. Plasma was not obtained from individuals (1) who were extreme exercisers (e.g., marathon runners), (2) with body-mass indices outside the 95th percentile, or (3) who adhered to extreme dietary regimens. Individuals should not have taken medication for at least 72 h prior to blood draw and were free from overt conditions (e.g., diabetes), diseases, and disorders.

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

Homogeneity Assessment: The homogeneity of all measurands for which certified and non-certified values are provided was assessed at NIST using the methods and test portion sizes described below; analysis of variance did not show statistically significant heterogeneity. The reported analytes have been treated as though they are homogeneously distributed in the material; the homogeneity of the other analytes present in the material and not reported by NIST and/or CDC was not assessed.

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