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National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 909c

Frozen Human Serum

This Standard Reference Material (SRM) is intended primarily for use in validating analytical methods for the determination of specified constituents in human serum. This SRM can also be used for quality assurance when assigning values to in-house control materials. A unit of SRM 909c consists of three vials, each containing 2 mL of frozen human serum.

Certified Concentration Values: Certified concentration values for selected constituents are provided in Table 1. 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]. The certified concentrations were determined using higher-order reference measurement procedures [2] calibrated with NIST high-purity neat or primary standard solution SRMs; the uncertainties are expanded uncertainties at the 95 % level of confidence [3]. The measurands are the constituents listed in Table 1 in human serum. The certified values are metrologically traceable to SI units for amount of substance and mass, expressed both in mass concentrations (milligrams per deciliter, micrograms per deciliter, nanograms per milliliter, or grams per liter) and amount concentration (nanomoles per liter or micromoles per liter).

Reference Concentration Values: Reference concentration values for total protein and transferrin glycoforms are provided in Table 2. A NIST reference value is a noncertified value that does not meet NIST criteria for certification and is provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty [1], or may reflect a lack of sufficient statistical agreement among multiple methods. The measurands are the constituents listed in Table 2 in human serum. The reference value for total protein is metrologically traceable to SI unit for mass, expressed in mass concentration (grams per liter) as realized by the method used. The reference values for transferrin glycoforms are metrologically traceable to SI unit for mass, expressed as mass fraction in percent, as realized by the methods used.

Expiration of Certification: The certification of **SRM 909c** is valid, within the measurement uncertainty specified, until **15 October 2025**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see "Instructions for Storage, Handling, 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.

Overall direction and coordination of the analyses was performed by K.W. Phinney of the NIST Biomolecular Measurement Division and S.E. Long of the NIST Chemical Sciences Division.

Acquisition and preparation of this SRM were coordinated by K.W. Phinney. Analytical measurements were performed by G. Ballihaut, T.A. Butler, J. Camara, W.C. Davis, S.E. Long, Y. Nuevo Ordóñez, K.W. Pratt, J.L. Prendergast, S.A. Rabb, L.T. Sniegoski, T.W. Vetter, M.J. Welch, and L.L. Yu of the NIST Chemical Sciences Division.

Consultation on the statistical design of the experimental work and evaluation of the data was provided by J.H. Yen and N.-F. Zhang of the NIST Statistical Engineering Division.

Support aspects involved with the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

Carlos A. Gonzalez, Chief
Chemical Sciences Division

Gaithersburg, MD 20899
Certificate Issue Date: 07 July 2017
Certificate Revision History on Last Page

Steven J. Choquette, Director
Office of Reference Materials

NOTICE AND WARNING TO USERS

SRM 909c IS INTENDED FOR LABORATORY USE ONLY. THIS IS A HUMAN SOURCE MATERIAL. HANDLE PRODUCT AS A BIOHAZARDOUS MATERIAL CAPABLE OF TRANSMITTING INFECTIOUS DISEASE. The supplier has reported that each donor unit of 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. However, no known test method can offer complete assurance that hepatitis B virus, hepatitis C virus, HIV, or other infectious agents are absent from this material. Accordingly, this human blood-based product should be handled at the Biosafety Level 2 or higher as recommended for any POTENTIALLY INFECTIOUS HUMAN SERUM OR BLOOD SPECIMEN in the Centers for Disease Control and Prevention/National Institutes of Health (NIH) Manual [4].

INSTRUCTIONS FOR STORAGE, HANDLING, AND USE

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

Handling and Use: SRM 909c is provided as frozen serum 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.

SOURCE, PREPARATION, AND ANALYSIS[(1)]

Source and Preparation: SRM 909c was prepared from "off-the-shelf" plasma that was then converted to serum by Aalto Scientific, Ltd. (Carlsbad, CA). There were no age or gender requirements for donors.

Analytical Approach for Determination of Cholesterol and Total Glycerides: Cholesterol was determined using the NIST isotope dilution gas chromatography – mass spectrometry (ID GC-MS) reference method [5,6]. This method is an approved higher-order reference measurement procedure according to the Joint Committee for Traceability in Laboratory Medicine (JCTLM) [7]. This procedure employs hydrolysis of cholesterol esters using potassium hydroxide in ethanol, followed by extraction with hexane, and derivatization of cholesterol using *bis*(trimethylsilyl)acetamide [6]. Cholesterol-25,26,27-$^{13}C_3$ was used as the internal standard. Total glyceride mass fractions were determined using the NIST ID GC-MS reference method described in reference [8] and approved by the JCTLM as a higher-order reference method. The method involves hydrolysis of triglycerides, 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 Creatinine: Creatinine was determined using an isotope dilution liquid chromatography – mass spectrometry (ID LC-MS) method [9] that is similar to a method [10] developed at the Laboratory of the Government Chemist (LGC) and is approved by the JCTLM as a higher-order reference measurement procedure.

Analytical Approach for Determination of Electrolytes: Chloride was determined using a NIST micro-coulometric titration method in which the chloride was reacted quantitatively with Ag^+ ion that was coulometrically generated at a silver anode. The data were corrected for the presence of other electro-active interferents, including bromide. Calcium, magnesium, and potassium were determined using an isotope dilution sector field inductively coupled plasma-mass spectrometry (ID ICP-MS) primary method involving spiking with stable isotopes of ^{42}Ca, ^{41}K and ^{26}Mg followed by oxidative equilibration using microwave dissolution [11]. Value assignment for sodium was based on the combined results from two analytical methods. The first consisted of an ion exchange gravimetric method [12] involving microwave digestion of the SRM 909c serum, followed by separation of the sodium fraction on a cation exchange column and addition of high-purity sulfuric acid to form Na_2SO_4. The amount of Na_2SO_4 was then gravimetrically determined after evaporation to dryness in a platinum crucible. A second method using inductively coupled plasma optical emission spectrometry (ICP-OES) was also used which consisted of microwave digestion of the serum with nitric acid. Manganese was used as an internal standard.

[(1)] Certain commercial instruments, materials, or processes are identified in this report 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 instruments, materials, or processes identified are necessarily the best available for the purpose.

Analytical Approach for Determination of Iron: Total iron was determined by isotope dilution inductively coupled plasma - mass spectrometry (ID ICP-MS). The method involved spiking the serum samples with ^{57}Fe, microwave digestion, and dilution with water.

Analytical Approach for Determination of Selenium: Total selenium was determined by isotope dilution inductively coupled plasma - mass spectrometry (ID ICP-MS). The method involved spiking the serum samples with ^{77}Se, microwave digestion, and dilution with butanol in water.

Analytical Approach for Determination of Transferrin and Transferrin Glycoforms: Total transferrin and transferrin glycoforms were determined by isotope dilution inductively coupled plasma - mass spectrometry (ID ICP-MS) [13]. The method involved saturation of the serum transferrin with iron and separation of individual transferrin glycoforms by SCX chromatography. Quantitation was based upon ID by either introducing a ^{57}Fe spike post column or directly spiking the serum samples with an in-house prepared transferrin spike in which ^{57}Fe had been bound to the protein. Transferrin glycoforms were determined by the mass fraction of each individual glycoform expressed as a percentage of the total transferrin.

Analytical Approach for Determination of Urea and Uric Acid: Urea was determined using a modification of the ID GC-MS method described in reference [14], approved by the JCTLM, in which the serum was spiked with urea-^{18}O, passed through a solid-phase extraction cartridge, concentrated, then derivatized to 6-methyluracil overnight. Uric acid was determined using a modification of the ID GC-MS method described in reference [15], approved by JCTLM as a higher-order reference method. Serum 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 N-(*t*-butyldimethylsilyl)-N-methyltrifluoroacetamide.

Analytical Approach for Determination of Total Protein: Total protein was determined using the JCTLM-approved reference method for total serum protein [16]. The measurements were calibrated using *SRM 927d Bovine Serum Albumin (7 % Solution)* as the reference standard.

Homogeneity Assessment: The homogeneity of all analytes was assessed at NIST using the methods and test portion sizes described above; analysis of variance did not show statistically significant heterogeneity.

Value Assignment: Each certified or reference value is the weighted mean of measurement means from each method used. The measured serum density is 1.024 12 g/mL with a standard deviation of 0.000 09 g/mL; this uncertainty was incorporated in values that are reported relative to units of volume.

Table 1. Certified Concentration Values in SRM 909c[a]

Analyte	Concentration (mmol/L)	Concentration (mg/dL)	Coverage Factor, *k*
Cholesterol	3.703 ± 0.081	143.2 ± 3.1	2
Creatinine	0.072 89 ± 0.001 61	0.824 5 ± 0.018 2	2
Total Glycerides	1.214 ± 0.017	107.5 ± 1.5[b]	2.1
Urea	4.321 ± 0.089	25.95 ± 0.53	2
Uric Acid	0.278 ± 0.006	4.68 ± 0.10	2
Calcium (Ca)	2.520 ± 0.027	10.10 ± 0.11	2.01
Chloride (Cl)	105.88 ± 0.21	375.37 ± 0.74	2
Magnesium (Mg)	0.895 3 ± 0.006 6	2.176 ± 0.016	2
Potassium (K)	4.160 ± 0.069	16.27 ± 0.27	2.18
Sodium (Na)	141.84 ± 0.25	326.10 ± 0.57	2
	(µmol/L)	(µg/dL)	
Iron (Fe)	16.18 0.70	90.34 ± 3.89	2.26
	(µmol/L)	(ng/mL)	
Selenium (Se)	1.503 ± 0.035	118.7 ± 3.3	2
		(g/L)	
Total Transferrin (Tf)		2.28 ± 0.11	2

[a] Each certified concentration value is the mean or combination of means of results provided by ID LC-MS, ID GC-MS, ID ICP-MS, ICP-OES, micro-coulometry and ion exchange-gravimetry. The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence, consistent with the ISO/JCGM Guide [3]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is the combined uncertainty that incorporates within-method uncertainty and Type B uncertainty components related to the analysis, and k is the coverage factor corresponding to approximately 95 % confidence for each analyte [3]. For estimates based on multiple methods, the combined uncertainty also incorporates between-methods uncertainty [17,18].

[b] Results in milligrams per deciliter are expressed as the equivalent concentration of triolein.

Table 2. Reference Concentration Values in SRM 909c[a]

Analyte	Concentration (g/L)	Coverage Factor, *k*
Total Protein	69.0 ± 2.0	2
	Mass Fraction[b] (%)	
Disialo Tf	1.61 ± 0.10	2
Trisialo Tf	3.93 ± 0.21	2
Tetrasialo Tf	76.93 ± 5.09	2
Pentasialo Tf	16.00 ± 4.84	2
Hexasialo Tf	1.71 ± 0.14	2

[a] The reference concentration value is the weighted mean of results from a single method. The uncertainty provided is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence, consistent with the ISO/JCGM Guide [3]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is the combined uncertainty that incorporates within-method uncertainty and Type B uncertainty components related to the analysis, and k is the coverage factor corresponding to approximately 95 % confidence for each analyte [3].

[b] Mass percent is based on total transferrin.

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Certificate Revision History: 07 July 2017 (Addition of iron, transferrin, and iron status marker constituents in SRM 909c; correction of the certified value for chloride; editorial changes) **25 August 2015** (Removal of glucose certified value; change of expiration date; editorial changes); **28 January 2015** (Addition of certified values for selected electrolytes, update and change of sodium value from reference to certified value, editorial changes); **16 April 2013** (Added traceability information for total protein determination); **28 October 2011** (Correction of reference value unit for total protein); **14 December 2010** (Original certificate issue 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) 948-3730; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.