National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 909c

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 bottles, 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 SRMs; the uncertainties are expanded uncertainties at the 95 % level of confidence [3]. The calibration provides direct traceability to the mole for the certified analytes in this SRM.

Reference Concentration Values: Reference concentration values for selected constituents 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, or may reflect a lack of sufficient statistical agreement among multiple methods [1].

Expiration of Certification: The certification of **SRM 909c** is valid, within the measurement uncertainty specified, until **15 October 2015**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see "Instructions for Handling, Storage, and Use"). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

The overall direction and coordination of the analyses was performed by K.W. Phinney of the NIST Biomolecular Measurement Division.

Acquisition and preparation of the SRM were coordinated by K.W. Phinney.

Analytical measurements were performed by G. Ballihaut, T.A. Butler, J. Camara, W.C. Davis, J.L. Prendergast, S.A. Rabb, L.T. Sniegoski, and M.J. Welch of the NIST Chemical Sciences Division.

Consultation on the statistical design of the experimental work and evaluation of the data were 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.

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Certificate Issue Date: 16 April 2013
Certificate Revision History on Last Page

Robert L. Watters, Jr., Director
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NOTICE AND WARNING TO USERS

SRM 909c IS INTENDED FOR IN-VITRO DIAGNOSTIC 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 HANDLING, STORAGE, AND USE

Storage: The SRM should be stored at –60 °C or lower in the original unopened vials. The certification does not apply to contents of previously opened vials as the stability of all analytes has not been investigated.

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 mass fractions were 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 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 [11,12]. This method is an approved higher-order reference measurement procedure according to the JCTLM. For certification of SRM 909c, this procedure was modified in that the serum was not passed through an ion exchange resin prior to concentration, freeze-drying, and derivatization.

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

Analytical Approach for Determination of Urea and Uric Acid: Urea was determined using a modification of the ID GC-MS method described in reference [13], 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 14, approved by JCTLM as a higher-order reference method. Serum samples were spiked with uric acid-$^{15}N_2$, mixed

[(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.

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 Sodium: Sodium was determined by inductively coupled plasma - optical emission spectrometry (ICP-OES). The method involves microwave digestion of the serum with nitric acid. Manganese was used as the internal standard.

Analytical Approach for Determination of Total Protein: Total protein mass concentration was determined using the JCTLM-approved reference method for total serum protein [15]. 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 mean of measurements from a single method. 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	Value (mmol/L)	Value (mg/dL)	Coverage Factor (*k*)
Cholesterol	3.703 ± 0.081	143.2 ± 3.1	2
Creatinine	0.072 89 ± 0.001 61	0.8245 ± 0.0182	2
Glucose	5.050 ± 0.059	90.98 ± 1.07	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

Analyte	Value (μmol/L)	Value (ng/mL)	Coverage Factor (*k*)
Selenium	1.503 ± 0.035	118.7 ± 3.3	2

[(a)] Each certified concentration value is the mean of results provided by ID LC-MS, ID GC-MS, or ID ICP-MS. The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence, consistent with the GUM [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 a coverage factor corresponding to approximately 95 % confidence for each analyte [3].

[(b)] Results in mg/dL are expressed as the equivalent concentration of triolein.

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

Analyte	Value (g/L)	Coverage Factor (*k*)
Total Protein	69.0 ± 2.0	2

	Value (mmol/L)	Value (mg/dL)	Coverage Factor (*k*)
Sodium	141.8 ± 0.2	326.1 ± 0.5	2.13

[(a)] Each reference concentration value is the mean of results from a single method. The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence, consistent with the GUM [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 a coverage factor corresponding to approximately 95 % confidence for each analyte [3].

REFERENCES

[1] May, W.; Parris, R.; Beck II, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definition of Terms and Modes Used at NIST for Value Assignment of Reference Materials for Chemical Measurements*; NIST Special Publication 260-136 (2000); available at http://www.nist.gov/srm/upload/SP260-136.PDF (accessed Apr 2013).

[2] NCCLS; *Development of Definitive Methods for the National Reference System for the Clinical Laboratory, Approved Guidelin*e; NCCLS Publication NRSCL 1-A; National Committee for Clinical Laboratory Standards: Wayne, PA (1991).

[3] JCGM 100:2008; *Evaluation of Measurement Data - Guide to the Expression of Uncertainty in Measurement*; (GUM 1995 with Minor Corrections), Joint Committee for Guides in Metrology (JCGM) (2008); available at http://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed Apr 2013); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297, U.S. Government Printing Office: Washington, DC (1994); available at http://www.nist.gov/pml/pubs/index.cfm (accessed Apr 2013).

[4] CDC/NIH; *Biosafety in Microbiological and Biomedical Laboratories, 5th ed*.; Richardson, J.; Barkley, W.E.; Richmond, J.; McKinney, R.W., Eds.; U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention and National Institutes of Health; US Government Printing Office: Washington, D.C. (2007); available at http://www.cdc.gov/biosafety/publications/index.htm (accessed Apr 2013).

[5] Ellerbe, P.; Meiselman, S.; Sniegoski, L.T.; Welch, M.J.; White V,E.; *Determination of Serum Cholesterol by a Modification of the Isotope Dilution Mass Spectrometric Definitive Method*; Anal. Chem., Vol. 61, pp. 1718–1723 (1989).

[6] Cohen, A.; Hertz, H.S.; Mandel, J.; Paule, R.C.; Schaffer, R.; Sniegoski, L.T.; Sun, T.; Welch, M.J.; White V, E.; *Total Serum Cholesterol by Isotope Dilution Mass Spectrometry: A Candidate Definitive Method*; Clin. Chem., Vol. 26, pp. 854–860 (1980).

[7] BIPM; JCTLM: *Joint Committee on Traceability in Laboratory Medicine*; http://www.bipm.org/en/committees/jc/jctlm/ (accessed Apr 2013).

[8] Ellerbe, P.; Sniegoski, L.T.; Welch, M.J.; *Isotope Dilution Mass Spectrometry as a Candidate Definitive Method for Determining Total Glycerides and Triglycerides in Serum*; Clin. Chem., Vol. 41, pp. 397–404 (1995).

[9] Dodder, N.G.; Tai, S.S.C.; Sniegoski, L.T., Zhang, N.-F.; Welch, M.J.; *Certification of Creatinine in a Human Serum Reference Material by GC-MS and LC-MS*; Clin. Chem., Vol. 53, pp. 1694–1699 (2007).

[10] Stokes, P.; O'Connor, G.; *Development of a Liquid Chromatography - Mass Spectrometry Method for the High-Accuracy Determination of Creatinine in Serum*; J. Chromatogr. B., Vol. 794, pp. 125–136 (2003).

[11] White V,E.; Welch, M.J.; Sun, T.; Sniegoski, L.T.; Schaffer, R.; Hertz, H.S.; Cohen, A.; *The Accurate Determination of Serum Glucose by Isotope Dilution Mass Spectrometry - Two Methods*; Biomed. Mass Spectrom., Vol. 9, pp. 395–405 (1982).

[12] Prendergast, J.L.; Sniegoski, L.T.; Welch, M.J.; Phinney, K.W.; *Modifications to the NIST Reference Measurement Procedure (RMP) for the Determination of Serum Glucose by Isotope Dilution Gas Chromatography/Mass Spectrometry*; Anal. Bioanal. Chem., Vol. 397, pp. 1779–1785 (2010).

[13] Welch, M.J.; Cohen, A.; Hertz, H.S.; Ruegg, F.C.; Schaffer, R.; Sniegoski, L.T.; White V.E.; *Determination of Serum Urea by Isotope Dilution Mass Spectrometry as a Candidate Definitive Method*; Anal. Chem., Vol. 56, pp. 713–719 (1984).

[14] Ellerbe, P.; Cohen, A., Welch, M.J., White V.E.; *Determination of Serum Uric Acid by Isotope Dilution Mass Spectrometry as a New Candidate Definitive Method*; Anal. Chem., Vol. 62, pp. 2173–2177 (1990).

[15] Doumas, B.T.; Bayse, D.D.; Carter, R.J.; Peters, Jr., T.; Schaffer, R.; *A Candidate Reference Method for Determination of Total Protein in Serum*; Clin. Chem., Vol. 27, pp. 1642–1650 (1981).

Certificate Revision History: 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.