



Certificate of Analysis

Standard Reference Material[®] 1952a

Cholesterol in Freeze-Dried Human Serum

(In Cooperation with the College of American Pathologists)

This Standard Reference Material (SRM) is intended primarily for use in evaluating the accuracy of procedures for the determination of cholesterol in serum, and in validating working or secondary reference materials. A unit of SRM 1952a consists of six 10 mL vials containing approximately 0.28 g of freeze-dried serum, two vials each at three different concentration levels of cholesterol. Before use, the serum in each vial must be reconstituted with 3.00 mL of distilled water.

Table 1. Certified Concentration Values for Cholesterol in Reconstituted SRM 1952a^(a)

Concentration Level	Molar Concentration (mmol/L)	Mass Concentration (mg/dL)
Low (1952a-1)	3.79 ± 0.07	146.4 ± 2.7
Medium (1952a-2)	5.93 ± 0.10	229.3 ± 4.0
High (1952a-3)	8.35 ± 0.14	322.9 ± 5.4

^(a) The measurand is the concentration level of cholesterol; metrological traceability is to the derived SI unit for concentration expressed as millimoles per liter or milligrams per deciliter.

Certified Values: 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 in Table 1 apply only to reconstituted serum at room temperature, 20 °C to 25 °C. The uncertainty in the certified value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence; it incorporates Type B uncertainty components related to the analyses, consistent with the ISO/JCGM Guide and with its Supplement 1 [2,3]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is the combined uncertainty, and the coverage factor, $k = 2$, corresponds to an approximately 95 % confidence for each analyte.

Expiration of Certification: The certification of **SRM 1952a** is valid, within the measurement uncertainty specified, until **30 September 2018**, provided the SRM is handled and stored in accordance with instructions given in this certificate (see “Instructions for Storage and Use”). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

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

Coordination of the technical measurements leading to the certification of this SRM was performed by M.J. Welch of the NIST Chemical Sciences Division and E. White V and K.W. Phinney of the NIST Biomolecular Measurement Division.

Certification measurements were performed by L.T. Sniegowski, S.S.-C. Tai, and M.J. Welch of the NIST Chemical Sciences Division and by P.M. Ellerbe, a NIST research associate at NIST through the College of American Pathologists.

Carlos A. Gonzalez, Chief
Chemical Sciences Division

Steven J. Choquette, Acting Director
Office of Reference Materials

Gaithersburg, MD 20899
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Statistical consultation for this SRM was provided by S.B. Schiller, K.R. Eberhardt, and N.F. Zhang of the NIST Statistical Engineering Division.

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

NOTICE AND WARNINGS TO USERS

SRM 1952a IS INTENDED FOR RESEARCH USE. THIS IS A HUMAN-SOURCE MATERIAL. HANDLE PRODUCT AS A BIOHAZARDOUS MATERIAL CAPABLE OF TRANSMITTING INFECTIOUS DISEASE. The supplier of the serum has tested the source materials used to prepare this product and found them non-reactive/negative for hepatitis B surface antigen (HbsAg) and human immunodeficiency (HIV) 1 and 2 antibodies. However, no known test method can offer complete assurance that hepatitis B 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 Manual [4].

INSTRUCTIONS FOR STORAGE AND USE

Storage: The freeze-dried serum should be stored at a temperature between 2 °C and 8 °C. It should not be frozen or exposed to sunlight or ultraviolet radiation.

Use: Remove the metal closure and lightly tap bottom of vial to dislodge any serum particles from the stopper. Carefully remove the stopper to avoid possible loss of serum particles. Use a Type I Class A volumetric transfer pipet or other dispenser of known accuracy to add 3.00 mL ± 0.01 mL of distilled water. The distilled water should be between 20 °C and 25 °C and should be added slowly to the sides of the vial while continually turning the vial. Any additional volume outside these limits will add to the specified uncertainty of the values in Table 1. Replace the stopper, swirl at intervals for approximately one hour, and finally invert the vial several times. Do not shake vigorously because this will cause frothing. Use immediately at temperatures between 20 °C and 25 °C, or store between 2 °C and 8 °C until ready for use, preferably within 8 h.

SOURCE, PREPARATION, AND ANALYSIS⁽¹⁾

Source and Preparation: The human serum used in the preparation of SRM 1952a was provided by the College of American Pathologists (CAP).

Analysis: The certified values for cholesterol were determined by isotope dilution gas chromatography–mass spectrometry (ID-GC-MS) [5,6]. This method is recognized as a higher-order reference measurement procedure by the Joint Committee for Traceability in Laboratory Medicine (JCTLM) [7]. Value assignment of the concentration of cholesterol in SRM 1952a was based on the results from the NIST ID-GC-MS reference measurement procedure for cholesterol in serum [5,6]. 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. The freeze-dried serum was reconstituted with distilled water as described in “Instructions for Storage and Use”. SRM 911c Cholesterol was used to calibrate the method, and cholesterol-25,26,27-¹³C₃ was used as the internal standard.

⁽¹⁾ Certain commercial instruments, materials, or processes are identified in this certificate to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the instruments, materials, or processes identified are necessarily the best available for the purpose.

REFERENCES

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- [2] 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 (2008); available at http://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed Feb 2016); 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 Feb 2016).
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Certificate Revision History: 11 February 2016 (Editorial changes); 05 December 2013 (Updated certified concentration values for cholesterol; material expiration date added; editorial changes); 08 January 1990 (Original certificate 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>.