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

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Standard Reference Material® 1951c
Lipids in Frozen Human Serum
CERTIFICATE OF ANALYSIS

Purpose: This Standard Reference Material (SRM) is intended primarily for use in evaluating the accuracy of clinical procedures for the determination of total cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, and total glycerides in human serum. It is also intended for use in validating working or secondary reference materials.

Description: A unit of SRM 1951c consists of four vials of frozen human serum, two vials each at two different analyte concentration levels. Each bottle contains approximately 1 mL of serum.

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]. Values for total cholesterol and total glycerides are based upon the means of the results from analyses at NIST and CDC using ID-GC-MS. The uncertainty provided with each 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 and expresses both the observed difference between the results from the methods and their respective uncertainties, consistent with the ISO/JCGM Guides [2,3]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is the combined uncertainty and k is a coverage factor corresponding to approximately 95 % confidence for each analyte [2]. For the certified values shown in Table 1, $k = 2$. The measurand is concentration value for each analyte listed in Table 1. Metrological traceability is to the SI derived unit for amount-of-substance concentration (expressed as millimoles per liter) and mass concentration (expressed as milligrams per deciliter). The certified values were determined at NIST and at the Lipid Reference Laboratory at the Centers for Disease Control and Prevention (CDC) by isotope dilution gas chromatography–mass spectrometry (ID-GC-MS) [4–7]. The methods used by NIST and CDC for measurement of total cholesterol and by NIST for measurement of total glycerides are recognized as higher-order reference measurement procedures by the Joint Committee for Traceability in Laboratory Medicine (JCTLM) [8]. The certified concentrations apply only to serum thawed to room temperature, 20 °C to 25 °C (see “Storage” and “Use”).

Table 1. Certified Values for SRM 1951c

Analyte	Amount-of-Substance Concentration		Mass Concentration	
	Level 1 (mmol/L)	Level 2 (mmol/L)	Level 1 (mg/dL)	Level 2 (mg/dL)
Total Cholesterol	3.943 ± 0.046	6.244 ± 0.072	152.44 ± 1.78	241.41 ± 2.80
Total Glycerides	1.717 ± 0.036	1.642 ± 0.036	152.0 ± 3.2[(a)]	145.4 ± 3.2[(a)]

[(a)] Total glycerides results are expressed as triolein in milligrams per deciliter.

Non-Certified Values: Non-certified values and additional information are provided in Appendix A.

Period of Validity: The certified values delivered by **SRM 1951c** are valid within the measurement uncertainty specified until **30 April 2030**. The certified values are nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

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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).

Safety: SRM 1951c IS INTENDED FOR RESEARCH USE. This is a human-source material. Handle product as a biohazardous material potentially capable of transmitting infectious disease. The supplier of the serum has reported that each donor unit of serum used in the preparation of this product has been tested by an FDA-approved method and found non-reactive/negative for hepatitis B surface antigen (HbsAg), human immunodeficiency (HIV) 1 and 2 antibodies, and hepatitis C virus (HCV). 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 as recommended by the Centers for Disease Control and Prevention/National Institutes of Health's Biosafety in Microbiological and Biomedical Laboratories for human-derived blood products where the presence of infectious agent(s) may be unknown [9].

Storage: The serum is shipped frozen (on dry ice), and upon receipt, should be stored frozen until ready for use. A freezer temperature of –20 °C is acceptable for storage up to one week. If a longer storage time is anticipated, the material should be stored at or below –60 °C. The SRM should not be exposed to sunlight or ultraviolet radiation. Storage of thawed material at room or refrigerator temperatures may result in changes in the analyte concentrations.

Use: Bottles of the SRM to be analyzed should be removed from the freezer and allowed to stand at room temperature until thawed. After the material is thawed to room temperature, it should be used **immediately**. The material should be swirled gently to mix it before aliquots are withdrawn.

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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 https://www.bipm.org/en/committees/jc/jcgm/publications (accessed Jan 2023); 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/tn1297/index.cfm (accessed Jan 2023).

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[8] JCTLM; available at http://www.bipm.org/en/committees/jc/jctlm/ (accessed Jan 2023).

[9] CDC/NIH; *Biosafety in Microbiological and Biomedical Laboratories, 6th ed.*; Meechan, P.; Hatcher, B.; Potts, J.; Eds.; U.S. Department of Health and Human Services, Public Health Service, CDC and National Institutes of Health; US Government Printing Office: Washington, D.C. (2009); available at https://www.cdc.gov/labs/BMBL.html (accessed Jan 2023).

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Certificate Revision History: 24 January 2023 (Change of period of validity; format changes; editorial changes); 05 January 2018 (Change of expiration date; editorial changes); 23 November 2015 (Editorial changes); 27 June 2013 (Original certificate issue 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*.

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APPENDIX A

Non-Certified Values: The non-certified values for HDL-cholesterol, LDL-cholesterol, and total cholesterol, provided by the Lipid Reference Laboratory at CDC, are provided in Table A1. Non-certified values are the best estimate of the true values based on available data; however, the values do not meet the NIST criteria for certification and are 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 analytical methods [1]. These results were obtained using CDC reference methods for lipids [10,11].

Values for HDL-cholesterol, LDL-cholesterol, and total cholesterol are based upon the means of results from analyses at CDC. The uncertainty provided with each non-certified value in Table A1 is an expanded uncertainty, *U*, at the 95 % level of confidence, and is calculated according to the method described in the ISO/JCGM Guide [2]. The measurand is the concentration value for each analyte listed in Table A1 as determined by the method or methods indicated. Metrological traceability is to the SI derived unit for mass concentration (expressed as milligrams per deciliter).

Table A1. Non-Certified Mass Concentration Values for HDL-Cholesterol, LDL-Cholesterol, and Total Cholesterol

Analyte	Level I (mg/dL)	Level II (mg/dL)
HDL-Cholesterol	41.0 ± 0.9	64.9 ± 1.7
LDL-Cholesterol	86.4 ± 1.4	143.8 ± 2.1
Total Cholesterol[(a)]	154.6 ± 1.1	244.8 ± 1.1

[(a)] Total cholesterol as determined by the Abell-Kendall reference method [10].

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).

Source of Material: SRM 1951c was prepared by Solomon Park Research Laboratories (Kirkland, WA) following a protocol developed by the Cholesterol Reference Materials Subcommittee of the National Committee for Clinical Laboratory Standards (NCCLS) [12]. The goal of the NCCLS project was to develop a commutable lipid reference material for total cholesterol that would be useful in most presently available field methods. A large-scale study of a prior lot of this material involving most of the major clinical measurement systems found no significant biases between results on that prior lot and those from fresh, unpooled serum. The study verified that material prepared following the recommendations of the NCCLS study is an appropriate mechanism for transferring accuracy from the definitive and reference methods to the clinical laboratories without significant matrix effects on the systems tested.

Preparation of Material: Donor units were collected and allowed to clot at room temperature for 4 h. The serum was removed from the clot and immediately cooled to approximately 4 °C. Each unit of donor serum was then analyzed for total cholesterol content to determine which donor units to pool. The donor units selected were then pooled. One-milliliter aliquots of the bulk pool were dispensed into 3 mL glass vials and frozen at –70 °C. This was accomplished within 50 h of the initial donor unit collection.

Analysis: Value assignment of the concentrations of total cholesterol and total glycerides (as triolein) was based on the combination of results from ID-GC-MS methods at NIST and CDC. In addition, CDC provided results for total cholesterol using the Abell–Kendall reference method [10]. Concentrations of HDL-cholesterol were determined at CDC using the ultracentrifugation reference method [11], and LDL-cholesterol was determined at CDC using the beta-quantification reference method [11].

Homogeneity Analysis: The homogeneity assessment was made at the time the certification analyses were performed. A stratified sampling plan was devised to test for homogeneity across the lot of vials. There was no apparent trend in the data when plotted against the sequence in which the vials were prepared.

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