National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1951b

Lipids in Frozen Human Serum

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 triglycerides (both total glyceride species and triglycerides only) in human serum. It is also intended for use in validating working or secondary reference materials. A unit of SRM 1951b consists of four bottles of frozen human serum, two bottles each of two different analyte concentration levels. Each bottle contains 1 mL of human serum.

Certified Concentration Values: The certified concentrations of total cholesterol and triglycerides were determined at NIST using isotope dilution/gas chromatography/mass spectrometry (ID/GC/MS) definitive methods [1]. The concentrations and their expanded uncertainties for the two concentration levels (SRM 1951b Level I and Level II) are listed in Table 1a in mmol/L and in Table 1b in mg/dL (as triolein for the triglycerides). The triglycerides concentrations are reported two ways: as total glycerides (the molar sum of free glycerol, monoglycerides, diglycerides, and triglycerides) and as triglycerides only. The certified concentrations apply only to serum thawed to room temperature, 20 °C to 25 °C (see "Instructions for Storage and Use").

Reference Concentration Values: Reference concentration values for HDL-cholesterol, LDL-cholesterol, total cholesterol, and triglycerides, provided by the Lipid Reference Laboratory at the Centers for Disease Control and Prevention (CDC), are reported in Table 2. These results were determined using CDC reference methods for lipids.

Expiration of Certification: The certification of **SRM 1951b** is valid, within the measurement uncertainty specified, until **31 December 2015**, provided the SRM is handled 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 Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before expiration, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

The overall direction and coordination of the analyses at NIST were under the chairmanship of M.J. Welch of the NIST Analytical Chemistry Division. The overall coordination of the measurements at CDC was under the direction of M.M. Kimberly of the CDC Lipid Reference Laboratory.

The analytical measurements at NIST were performed by L.T. Sniegoski, S.S.-C. Tai, and M.J. Welch of the NIST Analytical Chemistry Division. Analytical measurements at CDC were performed by S.H. Edwards and S. Stribling of the CDC Division of Laboratory Sciences.

The sampling protocol and statistical analysis of the data were performed by N.-F. Zhang of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Measurement Services Division.

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Analytical Chemistry Division

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Certificate Revision History on Last Page

Robert L. Watters, Jr., Chief
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NOTICE AND WARNINGS TO USERS

SRM 1951b 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 of this serum has reported that each donor unit of serum or plasma used in the preparation of this product was tested by an FDA-approved method and was found to be nonreactive for hepatitis B surface antigen (HbsAG), hepatitis C virus (HCV), and human immunodeficiency virus (HIV)-1 antibodies. 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 Center for Disease Control (CDC)/National Institutes of Health (NIH) Manual [2].

Stability: The material is kept at –80 °C for long term storage at NIST. Under these conditions, the analytes are expected to be stable.

INSTRUCTIONS FOR STORAGE AND USE

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.

SOURCE, PREPARATION, AND ANALYSIS[1]

Source of Material: SRM 1951b Lipids in Frozen Human Serum was prepared by the 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) under the chairmanship of G.L. Myers of the CDC [3]. 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 this 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 bottles and frozen at –70 °C. This was accomplished within 50 hours of the initial donor unit collection.

Analytical Methods: For the determination of the certified concentrations and uncertainties, a stratified sampling plan was devised to test for homogeneity across the manufacturing process. One group of samples was used for the determination of total cholesterol; a second set was used for the total glycerides and triglycerides. A method based on ID/GC/MS and considered to be a *definitive* method [1] for total serum cholesterol by the NCCLS was used for the determination of total cholesterol [4]. The total glycerides and triglycerides were determined using the NIST ID/GC/MS method for these analytes [5]. These methods are also recognized as approved higher-order reference measurement procedures by the Joint Committee on Traceability in Laboratory Medicine (JCTLM) [6].

HDL-cholesterol and LDL-cholesterol were determined at CDC using the betaquantification reference method [7] used in the Cholesterol Reference Method Laboratory Network (CRMLN). In addition, CDC provided results for total cholesterol using the Abell–Kendall reference method [7] and for total triglyceride (after subtraction of free glycerol) using the chromotropic reference method [7] used in the CRMLN.

[1]Certain commercial equipment, instruments, or materials 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 materials or equipment identified are necessarily the best available for the purpose.

Table 1a. Certified Concentration Values and Uncertainties for Analytes in SRM 1951b in mmol/L

Analyte	Level I (mmol/L)	Level II (mmol/L)
Total Cholesterol	4.804 ± 0.014	6.895 ± 0.022
Total Glycerides		2.988 ± 0.036
Triglycerides only	1.208 ± 0.013	2.700 ± 0.027

Table 1b. Certified Concentration Values and Uncertainties for Analytes in SRM 1951b in mg/dL

Analyte	Level I (mg/dL)[a]	Level II (mg/dL)[a]
Total Cholesterol	185.76 ± 0.55	266.58 ± 0.84
Total Glycerides		264.6 ± 3.2
Triglycerides only	107.0 ± 1.2	239.1 ± 2.4

[a] Total glycerides and triglycerides results are expressed as milligrams triolein per deciliter.

Each certified value is the mean of measurements made using definitive methods [4,5]. The expanded uncertainty, U, for each certified value is calculated from the equation, $U = ku_c$, where u_c is the combined standard uncertainty calculated according to the ISO Guide [8]. The coverage factor, $k = 2$, is determined from the Student's t-distribution corresponding to the calculated effective degrees of freedom and 95 % level of confidence.

Table 2. Reference Concentration Values and Uncertainties for HDL-Cholesterol, LDL-Cholesterol, Total Cholesterol, and Triglycerides

Analyte	Level I (mg/dL)[a]	Level II (mg/dL)[a]
HDL-Cholesterol	48.7 ± 0.9	51.9 ± 1.0
LDL-Cholesterol	113.2 ± 3.1	152.6 ± 3.0
Total Cholesterol[b]	187.4 ± 0.4	269.2 ± 0.6
Triglycerides	106.6 ± 0.5	239.8 ± 1.0

[a] Triglycerides results are expressed as milligrams triolein per deciliter.
[b] Total cholesterol as determined by the Abell-Kendall reference method [7]

The uncertainty in each reference value is for an individual measurement. It is expressed as an expanded uncertainty, U, at the 95 % level of confidence, and is calculated according to the method described in the ISO Guide [8]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of within-run variation and material inhomogeneity. The coverage factor, $k = 2$, is determined from the Student's t-distribution corresponding to the calculated effective degrees of freedom and 95 % level of confidence.

REFERENCES

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

[2] *Biosafety in Microbiological and Biomedical Laboratories*; U.S. Department of Health and Human Services; U.S. Government Printing Office: Washington, DC (1988).

[3] NCCLS; *Preparation and Validation of Commutable Frozen Human Serum Pools as Secondary Reference Materials for Cholesterol Measurement Procedures*; NCCLS Publication C37-A, National Committee for Clinical Laboratory Standards: Wayne, PA (2000).

[4] 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).

[5] 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).

[6] Joint Committee on Traceability in Laboratory Medicine (JCTLM) Home Page. http://www.bipm.org/en/committees/jc/jctlm/ (accessed Sep 2010).

[7] Myers, G.L.; Cooper, G.R.; Greenberg, N.; Kimberly, M.M.; Waymack, P.P.; Hassemer, D.J.; *Standardization of Lipid and Lipoprotein Measurements*. In: Rifai, N.; Warnick, G.R.; Dominiczak, M.H., Eds.; Handbook of Lipoprotein Testing, 2nd ed., AACC: Washington, D.C., pp. 717–748.

[8] JCGM 100:2008; *Evaluation of Measurement Data — Guide to the Expression of Uncertainty in Measurement* (ISO 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 Sep 2010); 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/physlab/pubs/index.cfm (accessed Sep 2010).

Certificate Revision History: 09 September 2010 (Removal of certified values in Level 1 for total glycerides because of instability; extension of expiration date; editorial changes); **14 March 2007** (Editorial changes); **03 August 2006** (Update of expiration date, addition of Table 2, and editorial changes); **14 May 2004** (This revision adds Levels I and II values for triglycerides and changes the material name); **03 February 2004** (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) 926-4751; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.