



# National Institute of Standards & Technology

## Certificate of Analysis

### Standard Reference Material<sup>®</sup> 1951b

#### Cholesterol 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 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 were determined at NIST using the ID/MS definitive method [1]. The concentrations and their expanded uncertainties for the two concentration levels (SRM 1951b Level I and Level II) are listed in Table 1 in mmol/L and in mg/dL. The certified concentrations apply only to serum thawed to room temperature, 20 °C to 25 °C; see "Instructions for Use".

**Expiration of Certification:** The certification of this SRM is valid until **31 December 2008**, within the measurement uncertainties specified, provided the SRM is handled and stored in accordance with the instructions given in the certificate. However, the certification is nullified if the SRM is damaged, contaminated, or 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 at NIST were under the chairmanship of M.J. Welch of the NIST Analytical Chemistry Division.

The analytical measurements at NIST were performed by L.T. Sniegowski and M.J. Welch of the NIST Analytical Chemistry Division.

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

The support aspects involved in the issuance of this SRM were coordinated through the NIST Standard Reference Materials Program by B.S. MacDonald of the NIST Measurement Services Division.

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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 HbsAG, HCV, and 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 CDC/National Institutes of Health (NIH) Manual [2].

**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 -50 °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.

**Stability:** The material is kept at -80 °C for long term storage at NIST. Under these conditions, the total cholesterol is expected to be stable. NIST will continue to monitor the stability of this analyte in this material and will notify purchasers of the material of any changes in the certified concentrations.

## INSTRUCTIONS FOR 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<sup>1</sup>

**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 [4]. 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 mL 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 Method:** For the determination of the certified concentrations and uncertainties, a stratified sampling plan was devised to test for homogeneity across the manufacturing process. A method based on isotope dilution/gas chromatography/mass spectrometry (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 [3]. This method is also recognized as an approved higher order reference measurement procedure by the Joint Committee on Traceability in Laboratory Medicine (JCTLM) [5].

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<sup>1</sup>Certain commercial equipment, instrumentation, or materials are identified in this certificate to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the NIST, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Table 1. Certified Concentrations<sup>a</sup> and Uncertainties for Total Cholesterol in SRM 1951b

Concentration Levels	Total Cholesterol Concentration	
	(mmol/L)	(mg/dL)
Level I	4.804 ± 0.014	185.76 ± 0.55
Level II	6.895 ± 0.022	266.58 ± 0.84

<sup>a</sup> Each certified value is the mean of measurements made using the definitive method [3]. The expanded uncertainty,  $U$ , for each certified value is calculated from the equation,  $U = k u_c$ , where  $u_c$  is the combined standard uncertainty calculated according to the ISO Guide [6] and  $k$  is a coverage factor.

#### REFERENCES

- [1] *Development of Definitive Methods for the National Reference System for the Clinical Laboratory, Approved Guideline*; NCCLS Publication NRSLC 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] Ellerbe, P.; Meiselman, S.; Sniegowski, 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).
- [4] *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).
- [5] Joint Committee on Traceability in Laboratory Medicine (JCTLM) Home Page. <http://www.bipm.fr/en/committees/jc/jctlm/> (accessed 14 January 2004).
- [6] *Guide to the Expression of Uncertainty in Measurement*, ISBN 92-67-10188-9, 1st Ed., ISO, Geneva, Switzerland (1993); 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://physics.nist.gov/Pubs/>.

*Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-6776; fax (301) 926-4751; e-mail [srminfo@nist.gov](mailto:srminfo@nist.gov); or via the Internet at <http://www.nist.gov/srm>.*