National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1951a

Lipids in Frozen Human Serum

Lot Nos. 95-LI and 95-LII

This Standard Reference Material (SRM) is intended primarily for use in evaluating the accuracy of clinical procedures for the determination of total cholesterol, HDL-cholesterol, 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 1951a 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 by definitive methods [1]. The concentrations and their expanded uncertainties for the two concentration levels (SRM 1951a 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 triglyceride concentrations are reported two ways: as total glycerides (the molar sum of free glycerol, monoglycerides, diglycerides, and triglycerides) and as triglycerides only. The associated combined uncertainties and degrees of freedom are shown in Table 2. The certified concentrations apply only to serum thawed to room temperature, 20 °C to 25 °C (see *Instructions for Use*).

Reference Concentration Values: Reference concentration values for total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, and glycerol blank, provided by the Lipid Reference Laboratory at the Centers for Disease Control and Prevention (CDC), are reported in Tables 3a and 3b. These results were determined using the CDC reference methods. The sum of these results for triglycerides and glycerol agree well with the NIST total glyceride concentrations in Table 1b.

Expiration of Certification: The certification of this SRM is valid from **30 September 1998 - Date Extended to 31 October 2005**, 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. Return of the attached registration card will facilitate notification.

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

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Certificate Issue Date: 10 July 2003
See Certificate Revision History on Page 5

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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 at CDC of the original HDL- and LDL-cholesterol analyses and stability analyses resulting in the revised reference values were under the direction of P.P. Waymack of the CDC Lipid Reference Laboratory.

The analytical measurements at NIST were performed by L.T. Sniegoski of the NIST Analytical Chemistry Division and P. Ellerbe of the College of American Pathologists (CAP), Research Associate at NIST. The analytical measurements at CDC were performed by C. Griffin, S. Ethridge, and R. Cheek of the CDC Lipid Reference Laboratory.

The sampling protocol and statistical analysis of the NIST data were performed by S.B. Schiller and M.G. Vangel of the NIST Statistical Engineering Division. Statistical analysis of the original CDC data was provided by S.J. Smith of CDC. Statistical analysis of the stability measurements performed at CDC was provided by N.F. Zhang of the NIST Statistical Engineering Division.

NOTICE AND WARNINGS TO USERS

SRM 1951a 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 and triglycerides are expected to be stable. NIST will continue to monitor the stability of these analytes 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[1]

Source of Material: SRM 1951a 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. 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 this material involving most of the major clinical measurement systems found no significant biases between results on this material and those from fresh, unpooled serum. The study verified that this material is an appropriate mechanism for transferring accuracy from the definitive and reference methods to the clinical laboratories.

[1]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.

Preparation of Material: Donor units were collected and allowed to clot at room temperature for four hours. 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 Methods: For the determination of the certified concentrations and uncertainties of the analytes (Tables 1a, 1b, and 2), 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 considered to be "definitive" [1] for total serum cholesterol by the NCCLS was used for the determination of total cholesterol [3]. A candidate definitive method was used for the determinations of total glycerides and triglycerides only [4]. Both methods used isotope dilution/gas chromatography/mass spectrometry. The material was found to be homogeneous for all analytes.

For the reference concentrations determined by the CDC (Tables 3a and 3b), total cholesterol was determined using the National Reference Method for total cholesterol [5,6]. Determination of HDL-cholesterol was performed with the CDC HDL reference procedure using ultracentrifugation to remove very-low-density lipoprotein (VLDL) that may interfere with the heparin-Mn^{2+} precipitation procedure [7]. LDL-cholesterol was measured indirectly using the beta quantification procedure [7]. Triglycerides were determined using the modified CDC reference method that corrects for free glycerol, phospholipids, and other potential interfering substances through extraction with methylene chloride and treatment with silicic acid [8]. Free glycerol was determined by using isotope dilution mass spectrometry [9].

Table 1a. Certified Concentrations[a] and Uncertainties for Analytes in SRM 1951a in mmol/L

Analyte	Level I (mmol/L)	Level II (mmol/L)
Total Cholesterol	4.7109 ± 0.0116	7.1554 ± 0.0142
Total Glycerides	1.1357 ± 0.0035	1.9477 ± 0.0066
Triglycerides only	1.0053 ± 0.0077	1.7462 ± 0.0107

Table 1b. Certified Concentrations[a] and Uncertainties for Analytes in SRM 1951a in mg/dL

Analyte	Level I (mg/dL)	Level II (mg/dL)
Total Cholesterol	182.15 ± 0.45	276.67 ± 0.55
Total Glycerides[b]	100.56 ± 0.31	172.46 ± 0.58
Triglycerides only[b]	89.01 ± 0.68	154.62 ± 0.95

[a] Each certified value is the mean of measurements made using the definitive method [3,4]. 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 [10] and k is a coverage factor.

[b] Total glycerides and triglycerides results are expressed as mg triolein per deciliter.

Table 2. Combined Standard Uncertainties[a] u_c, and Degrees of Freedom, df, for the Data in Tables 1a and 1b

	mmol/L		mg/dL		Degrees
	Level I	Level II	Level I	Level II	of Freedom (df)
Analyte	u_c	u_c	u_c	u_c	
Total Cholesterol	0.0049	0.0062	0.19	0.24	8
Total Glycerides	0.0015	0.0028	0.13	0.25	8
Triglycerides only	0.0034	0.0046	0.30	0.41	8

[a] The value u_c is intended to represent, at the level of one standard deviation, the uncertainty in mean concentration from the combined effect of measurement uncertainty and the uncertainty in the purity of the primary standards. The coverage factor, $k = 2.306$, is the Student's t-distribution for a 95 % confidence interval with eight degrees of freedom (df). Therefore, each certified value with its expanded uncertainty defines a range of values within which the true concentration is expected to lie with approximately 95 % confidence.

Table 3a. Reference Concentrations and Uncertainties[a] for Analytes in SRM 1951a in mmol/L Based Upon Results from the CDC Lipid Reference Laboratory

Analyte	Level I (mmol/L)	Level II (mmol/L)
Total Cholesterol	4.766 ± 0.010	7.252 ± 0.016
HDL-Cholesterol	1.240 ± 0.032	1.274 ± 0.048
LDL-Cholesterol	2.889 ± 0.064	4.83 ± 0.13
Triglycerides[b]	1.0962 ± 0.0095	1.893 ± 0.024
Free Glycerol[b, c]	0.0431 ± 0.0046	0.0632 ± 0.0033

Table 3b. Reference Concentrations and Uncertainties[a] for Analytes in SRM 1951a in mg/dL Based Upon Results from the CDC Lipid Reference Laboratory

Analyte	Level I (mg/dL)	Level II (mg/dL)
Total Cholesterol	184.30 ± 0.40	280.39 ± 0.60
HDL-Cholesterol	47.9 ± 1.2	49.3 ± 1.9
LDL-Cholesterol	111.7 ± 2.5	186.9 ± 4.9
Triglycerides[b]	97.06 ± 0.84	167.6 ± 2.1
Free Glycerol[b, c]	3.82 ± 0.41	5.60 ± 0.29

[a] The uncertainty in the 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 [10]. 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, is determined from the Student's t-distribution corresponding to the calculated effective degrees of freedom and 95 % level of confidence.

[b] Triglycerides and glycerol results are expressed as mg triolein per deciliter.

[c] Also referred to as "glycerol blank."

Most U.S. NIH funded epidemiological and interventional studies performed over the last 30 years have traced their total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglyceride values to the reference methods performed at the CDC Lipid Reference Laboratory. As shown in Tables 1a, 1b, 3a, and 3b, there is a small bias in the CDC reference method values for total cholesterol (about 1.2 % to 1.3 % high) compared to the NIST definitive method values. The slight bias appears to be due to a small amount of nonspecificity of the CDC Abell Kendall based reference method that has been discussed in some detail previously [11,12]. The National Cholesterol Education Program (NCEP) recommends that total cholesterol measurements be traceable to the CDC reference method [13,14]. Thus, NIST and the CAP anticipate that most manufacturers of total cholesterol reagents and calibrator materials, at least for distribution in the United States, will wish to use the CDC reference values, i.e., 184.30 mg/dL and 280.39 mg/dL for Levels I and II, respectively, and **NOT** the NIST definitive method values at this time.

For the measurement of triglycerides, manufacturers and laboratories that actually measure total glycerides i.e., no correction for free glycerol (also known as glycerol blank) should use the NIST certified values for total glycerides (i.e., 100.56 mg/dL and 172.46 mg/dL as triolein for Levels I and II, respectively). The CDC reference method for triglycerides appears to detect a small percentage of mono- and diglycerides that may be present in both reference materials and clinical specimens, but it does not detect free glycerol [4]. Therefore, those manufacturers and clinical laboratories that measure a glycerol blank or "net" triglyceride should use the CDC reference values for triglycerides (i.e., 97.06 mg/dL and 167.6 mg/dL for Levels I and II), respectively.

REFERENCES

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Certificate Revision History: 10 July 2003 (Appendix with reference values for glucose added); 18 September 2002 (This revision reflects an extension of the expiration date); 07 June 2001 (The concentrations for HDL-and LDL-cholesterol have been revised based on new stability data received from CDC); 24 November 2000 (This revision reflects an extension in the expiration date); 04 March 1999 (The concentrations for HDL- and LDL-cholesterol have been revised based upon new data received from CDC); 24 June 1997 (Original certificate date).

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; or via the Internet http://www.nist.gov/srm.

Appendix A

Reference Concentrations for Glucose in SRM 1951a

	mg/dL	mmol/L
Level 1	90.93 ± 0.93	5.047 ± 0.052
Level 2	91.37 ± 1.30	5.072 ± 0.072

Each reference concentration value was determined from measurements made at NIST using a method based on isotope dilution gas chromatography mass spectrometry [1]. The method used involves spiking a known mass of serum with a known mass of glucose-$^{13}C_6$. After isolation of the glucose from the serum, it is converted to a dibutylboronate acetate derivative for the GC/MS measurements. The instrument is calibrated using known mixtures of SRM 917b Glucose and the same glucose-$^{13}C_6$ used to spike the serum samples.

The uncertainty in the reference concentration is calculated as $U = ku_c$. The quantity u_c is the combined standard uncertainty, calculated according to the ISO guide [10], and accounts for the combined effect of the components of uncertainty. The coverage factor, *k,* is determined from the Student's *t*-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence level.

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