



# National Institute of Standards & Technology

## Certificate of Analysis

### Standard Reference Material<sup>®</sup> 911c

#### Cholesterol

This Standard Reference Material (SRM) is certified as a chemical of known purity. It is intended primarily for use in the calibration and standardization of procedures for the determination of cholesterol in research samples and for routine evaluations of daily working standards used in these procedures. A unit of SRM 911c consists of 2 g of material.

**Certified Value:** 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]. This certified value is the equally weighted mean of results obtained from the analytical methods. The expanded uncertainty in the certified concentration is calculated as  $U = ku_c$ . The quantity  $u_c$  is the combined standard uncertainty calculated based on a Bayesian approach in reference 1 and the ISO/JCGM Guide [2]. The coverage factor,  $k = 2$ , represents an approximate 95 % level of confidence. The measurand is the total mass fraction of cholesterol. Metrological traceability to the SI derived unit for mass fraction (expressed as percent).

Certified Cholesterol Mass Fraction: 99.2 %  $\pm$  0.4 %

**Reference Value:** The major impurity in this material is 5,24-cholestadiene-3 $\beta$ -ol, identified by nuclear magnetic resonance (NMR) analysis. The reference concentration value of this impurity is based upon measurements by liquid chromatography mass spectrometry (LC/MS) and liquid chromatography with ultra violet detection (LC/UV). A reference value is a noncertified value that is the best estimate of the true values; however, the value does not meet NIST criteria for certification and is provided with an associated uncertainty 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]. Based on the method used, the measurand is the mass fraction of 5,24-cholestadiene-3 $\beta$ -ol. Metrological traceability is to the SI derived unit for mass fraction (expressed as a percent).

Reference 5,24-Cholestadiene-3 $\beta$ -ol Mass Fraction: 0.72 %  $\pm$  0.13 %

This reference value is the equally weighted mean of results obtained from the analytical methods. The expanded uncertainty in the certified concentration is calculated as  $U = ku_c$ . The quantity  $u_c$  is the combined standard uncertainty calculated based on a Bayesian approach in reference 3 and ISO/JCGM Guide [2]. The coverage factor,  $k = 2$ , represents an approximate 95 % level of confidence.

**Expiration of SRM Certificate:** The certification of **SRM 911c** is valid, within the measurement uncertainty specified, until **31 December 2024**, 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 notification.

Overall direction and coordination of the technical measurements leading to the certification were provided by M.J. Welch of the NIST Chemical Sciences Division.

Carlos Gonzalez, Chief  
Chemical Sciences Division

Gaithersburg, MD 20899  
Certificate Issue Date: 11 May 2016  
*Certificate Revision History on Last Page*

Steven J. Choquette, Acting Director  
Office of Reference Materials

Analyses were performed at NIST by M.M. Schantz, L.T. Sniegowski, S. S-C Tai, M. Bedner, and M.J. Welch of the NIST Chemical Sciences Division and D.K. Hancock of the NIST Biochemical Sciences Division.

Statistical consultation was provided by 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.

## **SOURCE, PREPARATION AND ANALYSIS<sup>(1)</sup>**

The material was obtained from Sigma-Aldrich (St. Louis, MO) who performed a vacuum drying step on the material prior to shipping it to NIST.

Proton NMR was used to detect and identify impurities in the SRM. The primary impurity identified is 5,24-cholestadiene-3 $\beta$ -ol. In addition there are much smaller amounts of 5,25-cholestadiene-3 $\beta$ -ol and two other unidentified steroids.

Isotope dilution gas chromatography/mass spectrometry (ID/GS/MS) was used to compare the purity of SRM 911c with SRM 911b, the previous lot of this SRM [4]. Gas chromatography with flame ionization detection was performed using two different stationary phases. LC/MS and LC/UV were used to measure impurity levels, particularly 5,24-cholestadiene-3 $\beta$ -ol.

## **NOTICE AND WARNING TO USERS**

SRM 911c IS INTENDED FOR RESEARCH USE.

## **INSTRUCTIONS FOR STORAGE AND USE**

**Storage:** SRM 911c should be stored in a tightly-closed bottle at or below room temperature (–20 °C to 23 °C is recommended). It should not be subjected to heat, direct sunlight or sources of ultraviolet radiation. For extended periods of storage after opening, the material should be kept at or below room temperature in a desiccator under inert gas. It should be allowed to warm to room temperature before opening. If this procedure is followed, drying is unnecessary. Experience at NIST, where SRM 911a, a previous lot, was stored under inert gas at –15 °C, indicated that SRM 911c stored under the same conditions **may** be stable for as many as 10 years. If the purity of the material degrades beyond the limits certified, purchasers will be notified by NIST. If the material is stored in a refrigerator (2 °C to 8 °C), it is recommended that the material should not be used after three years from the date of shipment from NIST. If it is stored in the dark at room temperature, it is recommended that the material not be used after six months from the date of shipment from NIST.

**Preparation of Stock Standard Solution:** A stock standard solution of cholesterol in ethanol (5.00 mmol/L  $\pm$  0.02 mmol/L) may be prepared by dissolving 194.9 mg  $\pm$  0.1 mg of SRM 911c in 50 mL of warm absolute ethanol in a 100.0 mL volumetric flask, allowing the solution to cool, and diluting to exactly 100.0 mL with ethanol [5]. The 5.00 mmol/L solution of cholesterol in ethanol should be stored in an all-glass, tightly-stoppered bottle at 0 °C. Under such conditions this solution should be stable for about four months [6].

Solutions of cholesterol in glacial acetic acid gradually form cholesteryl acetate when stored and errors may result when using this solution [7].

All constituted solutions of cholesterol should be clear and display no turbidity.

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<sup>(1)</sup> 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.

## REFERENCES

- [1] May, W.; Parris, R.; Beck II, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*; NIST Special Publication 260-136; U.S. Government Printing Office: Washington, DC (2000); available at: <http://www.nist.gov/srm/publications.cfm> (accessed May 2016).
- [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 (JCGM) (2008); available at [http://www.bipm.org/utls/common/documents/jcgm/JCGM\\_100\\_2008\\_E.pdf](http://www.bipm.org/utls/common/documents/jcgm/JCGM_100_2008_E.pdf) (accessed May 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 May 2016).
- [3] Liu, H.K.; Zhang, N F.; *Bayesian Approach to Combining Results from Multiple Methods*; proceedings of the Section of Bayesian Statistical Science of American Statistical Society (2001).
- [4] 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).
- [5] *Fundamental of Clinical Chemistry*; Tietz, N, Ed.; W.B. Saunders Co.: Philadelphia, PA, p. 358 (1970).
- [6] Henry, R.D.; *Clinical Chemistry, Principles and Technics*; Hoeber Medical Division, Harper & Row: New York, p. 854 (1967).
- [7] Klein, B.; Kleinman, N.B.; *Esterification of Cholesterol in Glacial Acetic Acid*; *Clin. Chem.*, Vol. 20, pp. 90–91 (1974).

**Certificate Revision History:** 11 May 2016 (Updated storage information; editorial changes); 06 January 2016 (Editorial changes); 28 July 2014 (Extension of certification period, editorial changes); 12 May 2009 (Changed the “Instructions for Use” for the amount of cholesterol used in the stock standard solution from 193.7 mg to 194.9 mg  $\pm$  0.1 mg); 10 August 2007 (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 <mailto:srminfo@nist.gov>; or via the Internet at <http://www.nist.gov/srm>.*