National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 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 clinical 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 Purity and Uncertainty: 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 accounted for by NIST. 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 Guide [2]. The coverage factor, $k = 2$, represents an approximate 95 % level of confidence.

The purity and estimated uncertainty of SRM 911c is based upon scientific judgment and evaluation of the multiple analytical methods applied to this SRM in the certification process.

Certified Cholesterol Mass Faction: 99.2 % ± 0.4 %

Reference Value and Uncertainty: The major impurity in this material is 5,24-cholestadiene-3β-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.

5,24-Cholestadiene-3β-ol Mass Fraction: 0.72 % ± 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 [1] and ISO 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 uncertainties specified, until **31 December 2014**, provided the SRM is handled in accordance with instructions given in this certificate (see "Instructions for 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) will facilitate notification.

The overall direction and coordination of the technical measurements leading to the certification were provided by M.J. Welch of the Analytical Chemistry Division.

Stephen A. Wise, Chief
Analytical Chemistry Division

Gaithersburg, MD 20899
Certificate Issue Date: 12 May 2009
See Certificate Revision History on Last Page

Robert L. Watters, Jr., Chief
Measurement Services Division

Analyses were performed at NIST by M.M. Schantz, L.T. Sniegoski, S. S-C. Tai, M. Bedner, and M.J. Welch of the Analytical Chemistry Division and D.K. Hancock of the 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 Measurement Services Division.

Source of Material:[1] 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.

Purity Analyses: Proton NMR was used to detect and identify impurities in the SRM. The primary impurity identified is 5,24-cholestadiene-3β-ol. In addition there are much smaller amounts of 5,25-cholestadiene-3β-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 [3]. 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-3β-ol.

NOTICE AND WARNING TO USERS

WARNING: This SRM is for "in vitro" diagnostic use only.

Stability and Storage of this SRM: The SRM should be stored in a tightly-closed bottle at or below room temperature (–6 °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 −15 °C 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 91la 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.

INSTRUCTIONS FOR USE

Preparation of Stock Standard Solution: A stock standard solution of cholesterol in ethanol (5.00 mmol/L ± 0.02 mmol/L) may be prepared by dissolving 194.9 mg ± 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 [4]. 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 [5].

Solutions of cholesterol in glacial acetic acid gradually form cholesteryl acetate when stored and errors may result when using this solution [6].

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

[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.

REFERENCES

[1] 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).

[2] ISO; G*uide to the Expression of Uncertainty in Measurement*; ISBN 92-67-10188-9, 1st ed.; International Organization for Standardization: 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/.

[3] 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).

[4] *Fundamental of Clinical Chemistry*; N. Tietz, Ed., W.B. Saunders Co.: Philadelphia, PA, p. 358 (1970).

[5] Henry, R.D.; *Clinical Chemistry, Principles and Technics*; Hoeber Medical Division, Harper & Row: New York, p. 854 (1967).

[6] Klein, B.; Kleinman, N.B.; *Esterification of Cholesterol in Glacial Acetic Acid*; Clin. Chem., Vol. 20, pp. 90–91 (1974).

Certificate Revision History: 12 May 2009 (This editorial revision reports a change in the "Instructions for Use" for the amount of cholesterol used in the stock standard solution from 193.7 mg to 194.9 mg ± 0.1 mg); 10 August 2007 (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) 975-2200; fax (301) 926-4751; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.