NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY
U.S. DEPARTMENT OF COMMERCE

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Standard Reference Material® 911c
Cholesterol
CERTIFICATE OF ANALYSIS

Purpose: The certified value delivered by 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.

Description: 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 International System of Units (SI) derived unit for mass fraction (expressed as percent).

Certified Cholesterol Mass Faction: 99.2 % ± 0.4 %

Non-Certified Value: A non-certified value is provided in Appendix A.

Additional Information: Additional information is provided in Appendix B.

Period of Validity: The certified value delivered by **SRM 911c** is valid within the measurement uncertainty specified until **31 December 2034**. The certified value is nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

Maintenance of Certified Value: NIST will monitor this SRM over the period of its validity. If substantive technical changes occur that affect the certification, NIST will issue an amended certificate through the NIST SRM website (https://www.nist.gov/srm) and notify registered users. SRM users can register online from a link available on the NIST SRM website or fill out the user registration form that is supplied with the SRM. Registration will facilitate notification. Before making use of any of the values delivered by this material, users should verify they have the most recent version of this documentation, available through the NIST SRM website (https://www.nist.gov/srm).

Carlos A. Gonzalez, Chief
Chemical Sciences Division

Steven J. Choquette, Director
Office of Reference Materials

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Safety: SRM 911c IS INTENDED FOR RESEARCH 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 ± 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 [3]. 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 [4].

Solutions of cholesterol in glacial acetic acid gradually form cholesteryl acetate when stored and errors may result when using this solution [5].

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

REFERENCES

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

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

[6] 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).

[7] 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).

Certificate Revision History: 30 September 2024 (Change of period of validity; updated format; editorial changes); 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 ± 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 Office of Reference Materials 100 Bureau Drive, Stop 2300, Gaithersburg, MD 20899-2300; telephone (301) 975-2200; e-mail srminfo@nist.gov; or the Internet at https://www.nist.gov/srm.

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APPENDIX A

Non-Certified Value: The major impurity in this material is 5,24-cholestadiene-3β-ol, identified by nuclear magnetic resonance (NMR) analysis. The non-certified 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 non-certified value 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].

Non-Certified 5,24-Cholestadiene-3β-ol Mass Fraction: 0.72 % ± 0.13 %

This non-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 6 and ISO/JCGM Guide [2]. The coverage factor, $k = 2$, represents an approximate 95 % level of confidence.

Period of Validity: The non-certified value is valid within the measurement uncertainty specified until **31 December 2034.** The value assignment is nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

Maintenance of Non-Certified Value: NIST will monitor this material to the end of its period of validity. If substantive technical changes occur that affect the non-certified value during this period, NIST will update this Appendix and notify registered users. SRM users can register online from a link available on the NIST SRM website or fill out the user registration form that is supplied with the SRM. Registration will facilitate notification. Before making use of any of the values delivered by this material, users should verify they have the most recent version of this documentation, available through the NIST SRM website (https://www.nist.gov/srm).

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APPENDIX B

Source, Preparation, and Analysis: 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-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 [7]. 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.

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