U. S. Department of Commerce
Alexander B. Trowbridge,
Secretary

National Bureau of Standards
A. V. Astin, Director

Certificate of Analysis

Standard Reference Material 911
Cholesterol

This Standard Reference Material is certified as a chemical standard to aid manufacturers in meeting the specifications for cholesterol for use in clinical analysis.

This cholesterol was purified according to the method of L. F. Fieser [J. Am. Chem. Soc. 75, 5421 (1953)], which consists of preparing cholesterol dibromide, regenerating the cholesterol by reacting the dibromide with zinc dust, and crystallizing. The purity is 99.4 ± 0.3 percent.

Purity was determined by gas chromatography, thin-layer chromatography, and mass, infrared, and nuclear magnetic resonance spectroscopy. Gas-chromatographic examination, using Gas–Chrom Q treated with 3 percent OV–17 as the stationary phase at 240°C, showed impurity peaks at the following retention times relative to cholesterol:

Relative Retention Time	Area Relative to Cholesterol Peak (Percent)
0.5	0.1
1.2	0.3
1.6	0.1
1.8	0.1

Thin-layer chromatography on silica gel developed with chloroform, sprayed with sulfuric acid in methanol, and heated to 110° revealed two trace contaminants under ultraviolet irradiation. The greater contaminant appeared at the head of the cholesterol; the lesser, just beyond the origin. By two-dimensional chromatography, the greater contaminant was found as a separate spot. No quantitative values were assigned to these spots.

Response to the Liebermann–Burchard test was very uniform. A molar absorbance of 590 ± 1 at 535 nm was obtained by using 2.9 ml of 0.1 percent (wt/vol) cholesterol solution in glacial acetic acid and 9.96 ml of reagent prepared from 300 ml of acetic anhydride, 150 ml of acetic acid, 50 ml of sulfuric acid, and 10 g of sodium sulfate. The volumes in each analysis were calculated from the weights of each solution delivered and the densities. Measurements of absorbance, with the reaction performed at 25°C, were obtained 40 minutes after mixing. Molar absorbances higher than 590 could be obtained with mixtures containing less acetic acid, but the objective was to obtain high reproducibility and not to maximize the molar absorbance [Ness, Pastewka, and Peacock, Clin. Chim. Acta 10, 229 (1964)]. All measures of precision used in this certificate are estimated at the 95 percent confidence level.

WASHINGTON, D. C. 20234
October 20, 1967

W. Wayne Meinke, Chief
Office of Standard Reference Materials

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The melting point of this material is 148.8 ± 0.1°C at a pressure of less than 1 mm of Hg. This value was obtained by using a modification of the technique described by Enagonio, Pearson, and Saylor [Temperature, Its Measurement and Control in Science and Industry, Vol. III, Part 1, pp. 219–230. The Reinhold Publishing Co., New York, N.Y. (1962)]. Temperatures were measured by use of a platinum resistance thermometer calibrated in accordance with the International Practical Temperature Scale (1948).

The capillary melting point is 148.7°C (corrected). The melting point is that temperature at which the last trace of crystalline cholesterol melted. The cholesterol was under a pressure of less than 1 mm of mercury, and was heated at the rate of 0.5°C per min. Solidification of the melt followed by remelting gives this same value repeatedly.

The specific rotation, $[\alpha]^{20}D$, is –40° (c 2.241, $CHCl_3$). Microchemical analysis found 83.86 ± 0.08 wt percent carbon and 11.98 ± 0.09 wt percent hydrogen. The theoretical percentages based on $C_{27}H_{46}O$ are 83.87 wt percent carbon and 11.99 wt percent hydrogen.

Mass spectroscopy revealed the presence of contaminants having masses 14 and 28 units greater than that of the cholesterol parent peak. Infrared and nuclear magnetic resonance spectroscopy did not reveal the presence of any significant impurities. The selenium dioxide test for lathosterol was negative [Fieser, J. Am. Chem. Soc. 75, 4395 (1953)].

Preliminary indications of storage life were measured by storing a sample under vacuum at 80°C for one month. No changes were noted in melting point or infrared spectrum. The purity of the cholesterol will be followed over a 2-year period. If these additional measurements indicate a significant departure from the purity as stated, users of this material will be notified.

The Standard Reference Cholesterol is packaged in 0.5–g quantities sealed under nitrogen in screw–cap vials.

Analyses were conducted by A. Cohen, D. H. Freeman, R. T. Leslie, R. A. Paulson, C. B. Romaine, R. Schaffer, and C. L. Stanley of the NBS Institute for Materials Research, and H. F. Fales of the National Institutes of Health.