National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1845

Cholesterol in Whole Egg Powder

This Standard Reference Material (SRM) is intended primarily for use in evaluating the reliability of analytical methods used for the determination of cholesterol in whole egg material and similar food, and biological materials. SRM 1845 consists of one glass bottle, containing approximately 35 g of dried whole egg powder. SRM 1845 is one of a number of NIST reference materials available for evaluating the role of cholesterol in health and disease, establishing dietary requirements and recommendations for cholesterol, and accumulating accurate base-line and concentration data for cholesterol in foods.

Certified Cholesterol Concentration: The cholesterol concentration, expressed as a mass fraction in g/kg (mg/g) on an as-received basis, was determined at NIST using a modification of the isotope dilution mass spectrometric (IDMS) definitive method for cholesterol [1]. 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. A certified value is the present best estimate of the "true" value based on the results of analyses performed at NIST and collaborating laboratories.

Certified Cholesterol Concentration and Uncertainty

18.64 g/kg ± 0.39 g/kg

The certified concentration value and the uncertainty apply to a minimum sample size of 60 mg of the undried material. The uncertainty in the certified value, calculated according to the method described in the ISO Guide [2], is expressed as an expanded uncertainty, U. 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-method components of uncertainty and a component for observed material variability between bottles. The coverage factor, $k = 2$, corresponds to approximately 95 % confidence for each analyte.

Expiration of Certification: The certification of **SRM 1845** is valid, within the measurement uncertainties specified, until **11 June 2009**, 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 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 of the certification and stability testing were under the chairmanship of E.V White and M.J. Welch of the NIST Organic Analytical Research Division.

Cholesterol was determined by L.T. Sniegoski and M.J. Welch of the NIST Organic Analytical Research Division, and P. Ellerbe and S.S-C. Tai, Research Associates of the College of American Pathologists at NIST.

Stephen A. Wise, Chief
Analytical Chemistry Division

Gaithersburg, MD 20899
Certificate Issue Date: 17 August 2009
See Certificate Revision History on Last Page

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

The statistical analysis of the original certification data was performed by R.C. Paule of the NIST National Measurement Laboratory. The statistical analysis of the revised data was reviewed by N.F. Zhang of the NIST Statistical Engineering Division.

Support aspects involved in preparation, certification, and issuance of this SRM were originally coordinated through the NIST Standard Reference Materials Program by W.R. Wolf, NIST/USDA Research Associate, and R. Alvarez.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Measurement Services Division.

INSTRUCTIONS FOR USE

WARNING: For "in vitro" use only. **NOT** for human consumption.

Use: Allow bottle to come to room temperature before opening. **DO NOT dry the sample before use**. A minimum sample size of 60 mg must be used for the certified value and uncertainty to be valid. Smaller sample sizes may give values outside of the specified limits. The exposure of the sample to air should be minimized and any unused portions should be stored in a sealed bottle (see "Storage").

Storage: The whole egg powder, as received, should be stored in a refrigerator at a temperature between 2 °C and 8 °C. It should not be exposed to sunlight or ultraviolet light.

Stability: The stability of this SRM has been monitored and the cholesterol concentration has degraded slightly since it was originally certified. Monitoring will continue and, if significant changes in the concentration are observed, the purchaser will be notified. Registration (see attached sheet) will facilitate notification.

PREPARATION AND ANALYSIS[1]

Preparation: The whole egg powder material used in SRM 1845 was obtained, prepared, and homogenized by Agriculture Canada as previously described [3]. This material consists of Grade A (Canada) chicken eggs, dried with color and maximum 2 % Zeolex (sodium silico aluminate) added as an anticaking agent. The material was radiation-sterilized in bulk and subsequently packaged in a Class 100 clean air hood at NIST.

Analysis: The certified concentration for cholesterol in SRM 1845 is traceable to the International System of Units (SI) by use of a primary ratio method, isotope dilution mass spectrometry [1], calibrated with a primary reference compound, SRM 911b Cholesterol. The following description of the sample preparation and the analytical methods provides the user of this material with more information on the specific procedures used for certification. This information is given to encourage further examination of various analytical methodologies used.

A total of thirteen cholesterol measurements on samples from nine individual bottles were carried out in three independent sets of analyses. In each set, two samples of SRM 909 *Human Serum* were analyzed as controls.

The whole egg powder samples were prepared as follows. An accurately known amount of cholesterol-25,26,27$^{13}C_3$ of about 1 mg in 1 mL of ethanol was placed in a 50-mL standard tapered round-bottomed flask. An accurately weighed amount of whole egg powder (60 mg) was added. After addition of 15 mL of reagent alcohol (ethanol/methanol/2-propanol, 90/5/5 by volume) and 3 mL of potassium hydroxide solution (3 g KOH to 2 mL H_2O), the mixture was refluxed in a boiling water bath for 1 h, using a water-cooled condenser. The mixture was cooled and transferred to a 125-mL separatory funnel. Then 15 mL of water was added and the cholesterol was extracted with 30 mL of hexane. The hexane layer was washed four times with 5-mL portions of water, transferred to beaker, and allowed to evaporate in a hood. The residue was taken up in about 1 mL of methanol. A 0.1 mL aliquot was dried in a Reacti-Vial and then derivatized with 0.1 mL BSA[N,O-bis(trimethyl-silyl)acetamide].

[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.

The hydrolysis procedure was based upon the method given in Reference 2. The appropriate hydrolysis time was determined by spiking a whole egg powder sample, hydrolyzing for 15, 30, 60, 90, 120, and 180 min, extracting, derivatizing, and analyzing aliquots by gas chromatography mass spectrometry (GC/MS). No difference in the ratio of labeled to unlabeled cholesterol was observed in any of these samples and 60 min was chosen as the time for hydrolysis. A preliminary acid hydrolysis recommended for dried whole egg solid was not done because decomposition of cholesterol occurred as shown by adding labeled cholesterol to samples before and after acid hydrolysis.

The preparation, spiking, hydrolysis, extraction, and derivatization of SRM 909 *Human Serum* samples used as controls and the measurement of all samples by GC/MS followed the published definitive method for cholesterol [1].

Confirmatory measurements to test for evidence of bias in the measurement process were performed on a subset of samples at the conclusion of the principal measurements. The confirmatory measurements were performed two ways: (1) monitoring electron impact fragment ions at m/z 329 and 332, and (2) monitoring the $(M + NH_4\text{-}TMSOH)^+$ ions formed by ammonia chemical ionization.

The primary measurements were in excellent agreement over the three sets of analyses performed. The confirmatory measurements differed from the principal measurement by slightly more than has been observed for the control material (SRM 909); however, the differences were small relative to the inhomogeneity of the material. For determination of the final certified value, no data were rejected. The SRM 909 samples run in each set were in control.

REFERENCES

[2] Ellerbe, P.; Meiselman, S.; Welch, M.J.; White V, E.; *Presented at the 34th Annual Conference on Mass Spectrometry and Allied Topics*; Cincinnati, OH (1986).

[3] JCGM 100:2008; *Guide to the Expression of Uncertainty in Measurement*; (ISO GUM 1995 with Minor Corrections), Joint Committee for Guides in Metrology: BIPM, Sèvres Cedex, France (2008); available at http://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf; 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/.

[1] Ihnat, M.; *Fresenius' Zeitschrift fur Analytische Chemie*; Vol. 332, pp. 539–545 (1988).

Certificate Revision History: 17 August 2009 (The certification period has been shortened due to deterioration of the material); 13 March 2007 (Update of expiration date and editorial changes) 11 February 2003 (Certified cholesterol value and expiration date updated); 25 April 1994 (Unit size changed to 35 g); 09 January 1989 (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.