U. S. Department of Commerce
C. William Verity
Secretary

National Bureau of Standards
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National Bureau of Standards

Certificate of Analysis

Standard Reference Material 1952

Cholesterol in Human Serum (Freeze-Dried)

(In Cooperation with the College of American Pathologists)

This Standard Reference Material (SRM) is intended for use in evaluating the accuracy of clinical procedures for the determination of cholesterol in serum, in calibrating instruments and equipment used in these procedures, and in validating working or secondary reference materials. SRM 1952 consists of six vials of freeze-dried serum, two each of three different concentration levels of cholesterol. The serum was provided by the College of American Pathologists(CAP).

WARNING: FOR IN VITRO DIAGNOSTIC USE ONLY

HANDLE AS IF CAPABLE OF TRANSMITTING DISEASE!

FOLLOW STORAGE INSTRUCTIONS

CERTIFIED CHOLESTEROL CONCENTRATIONS: Cholesterol was determined at NBS by a modification [1] of the isotope dilution mass spectrometric definitive method [2]. The certified concentrations are given in two ways, corresponding to whether reconstitution is done with or without weighing the freeze-dried serum contents of a vial. Concentration values having smaller uncertainties may be obtained by weighing both the freeze-dried serum and the added water for reconstitution as described in Procedure A. In this case, the certified values in concentration per gram of freeze-dried serum, which appear in Table 1, should be used. When the contents of a vial are not weighed as in Procedure B , the values for the certified concentrations and their uncertainties in Table 2 should be used. These values apply to all vials of reconstituted SRM 1952. The user is advised to consider carefully the instructions provided in this certificate before selecting the appropriate table for use.

Table 1. Certified Cholesterol Concentrations and Uncertainties per Gram of Freeze-Dried Serum after Reconstitution of SRM 1952 according to Procedure A.

Concentration Level	Concentration per gram ±uncertainty, $mmol \cdot L^{-1} \cdot g^{-1}$	Concentration per gram ± uncertainty $mg \cdot dL^{-1} \cdot g^{-1}$	Number of Vials
Low (1952-1)	22.19 ± 0.11	858.1 ± 4.3	8
Medium (1952-2)	28.22 ± 0.09	1091.3 ± 3.6	9
High (1952-3)	35.79 ± 0.12	1383.9 ± 4.8	9

Notes

1. The certified concentrations apply to reconstituted serum at room temperature (20 to 25 °C).
2. The uncertainties are given as 99% confidence intervals.
3. Number of vials analyzed are as indicated. One sample from each vial was measured twice according to the protocol in Ref. 1.

Gaithersburg, MD 20899
February 29, 1988

Stanley D. Rasberry, Chief
Office of Standard Reference Materials

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Table 2. Certified Cholesterol Concentrations and Uncertainties in Reconstituted SRM 1952 for Use with Procedure B.

Concentration Level	Concentration and Uncertainty, mmol/L	Concentration and Uncertainty, mg/dL	Number of Vials
Low (1952-1)	5.34 ± 0.10	206.6 ± 3.9	8
Medium (1952-2)	6.77 ± 0.12	261.8 ± 4.6	9
High (1952-3)	9.23 ± 0.17	356.7 ± 6.4	9

Notes

1. The certified concentrations apply to reconstituted serum at room temperature (20-25 °C).
2. The uncertainties are 95%/95% statistical tolerance intervals and reflect the combined effects of measurement imprecision and the variability of the mass of dry serum among vials. The intervals are constructed so that, at a confidence level of 95%, they will include the concentrations for 95% of all vials of SRM 1952, when reconstituted according to Procedure B.
3. Number of vials analyzed are as indicated. One sample from each vial was measured twice according to the protocol in Ref. 1.

Cholesterol was determined by L.T. Sniegoski, NBS Organic Analytical Research Division, and P.M. Ellerbe, NBS Research Associate, College of American Pathologists. Distribution of fill weights among sample vials by refractive index was determined by R.G. Christensen, NBS Organic Analytical Research Division.

The statistical analysis of the data was performed by S.B. Schiller and K.R. Eberhardt of the NBS Statistical Engineering Division. Overall direction and technical measurements leading to the certification were under the chairmanship of M.J. Welch, E. White V., and R. Schaffer, NBS Organic Analytical Research Division. Technical and support aspects involved in the preparation, certification, and issuance of this Standard Reference Material were coordinated through the Office of Standard Reference Materials by R. Alvarez.

INSTRUCTIONS FOR USE

Two procedures for reconstituting SRM 1952 are described. Selection of a procedure depends on the uncertainties required for the concentration of cholesterol. If smaller uncertainties than those shown in Table 2 are required, the freeze-dried serum contents of a vial and the added water must be weighed as described in Procedure A.

Procedure A. Reconstitution with weighing of the freeze-dried serum: Completely remove label and adhesive by scraping the vial and then wiping it with a tissue moistened with a solvent, such as acetone or ethanol. Scratch an identification on vial. Remove metal closure and lightly tap bottom of vial to dislodge any serum particles adhering to the stopper. Dislodge stopper to equalize air pressure, then replace stopper, wipe surface of vial, and weigh to the nearest 0.1 mg. Remove stopper carefully to avoid possible loss of serum particles. Use a Type 1 Class A volumetric transfer pipet or other dispenser of known accuracy to slowly add 3.00 ± .01 mL of distilled water at a known temperature between 20-25 °C to the sides of the vial while continually turning the vial. Replace stopper and reweigh to obtain mass of water. Swirl vial at intervals for approximately one hour and finally invert the vial several times. Do not shake vigorously because this will cause frothing. Total time for reconstitution is approximately one hour. After reconstitution, use contents as soon as possible. If not used immediately, store between 2 and 8 °C until ready for use, preferably within 8 hours. After the reconstituted serum has been used, clean and dry the vial and its stopper. Reweigh after replacing stopper. The mass of dry serum is given by the difference between the original and final weighings.

The volume, V, of water actually added, is calculated by dividing the mass of water by the density of water at the known temperature.

After the contents of a vial are weighed and reconstituted with water, the concentration of cholesterol in this vial is calculated by multiplying the mass of freeze-dried serum, in grams, by the certified concentration of cholesterol per gram of freeze-dried serum (given in Table 1). However, if the volume of water added is not exactly 3.00 mL, the concentration of the reconstituted serum will be less or greater than the certified concentration, depending on whether too much or too little water was added. Therefore, another factor, representing the ratio of the nominal amount of water added (3.00 mL) to the actual volume of water added (V) must be multiplied by the calculated concentration to obtain the appropriate value for comparison. This factor is:

$$VR = \frac{3.00}{V}$$

For example, if the freeze-dried serum in the high cholesterol concentration level vial weighs 0.2590 g, and the volume of water added by weight was 2.98 mL, the certified concentration of cholesterol in this vial would be:

$$35.79 \text{ mmol}\cdot L^{-1}\cdot g^{-1} \times 0.2590 \text{ g} \times \frac{3.00 \text{ mL}}{2.98 \text{ mL}} = 9.33 \text{ mmol/L}$$

The uncertainty is calculated similarly and for this example becomes

$$\pm 0.12 \text{ mmol}\cdot L^{-1}\cdot g^{-1} \times 0.2590 \text{ g} \times \frac{3.00 \text{ mL}}{2.98 \text{ mL}} = \pm 0.031 \text{ mmol/L}$$

Procedure B. Reconstitution of SRM 1952 without weighing freeze-dried serum: Remove metal closure and lightly tap bottom of vial to dislodge any serum particles on stopper. Carefully remove stopper to avoid possible loss of serum particles. As described in Procedure A, reconstitute with 3.00 ± 0.01 mL of distilled water. Any additional volume outside these limits will add to the specified uncertainty of the values in Table 2. Use immediately at between 20-25 °C or store between 2 and 8 °C until ready for use, preferably within 8 hours.

The uncertainties for Procedure B reflect the combined effects of measurement imprecision and the variability of the mass of dry serum among vials. For the latter, tolerance intervals covering 95% of the serum dry weights with 96% confidence were computed for the mass of dry serum in the vials. These tolerance intervals are:

Concentration Level	Tolerance Intervals for Dry Mass, mg	Mean, mg
Low (1952-1)	237.4 - 244.0	240.7
Medium (1952-2)	236.5 - 243.4	239.9
High (1952-3)	254.0 - 261.5	257.8

For each concentration level, the tolerance interval for the dry mass is combined with the confidence interval for cholesterol concentration per gram from Table 1 to get a tolerance interval for the cholesterol concentration in the reconstituted serum. The limits and center of the new interval are the product of the respective limits and centers of the mass and cholesterol concentration per gram intervals. The results of these calculations are shown in Table 2.

STORAGE: The freeze-dried serum should be stored at a temperature between 2 and 8 °C. It should not be frozen or exposed to sunlight or ultraviolet radiation. Under the recommended storage conditions, this SRM is expected to be stable for at least two years. Should evidence indicate degradation, purchasers will be notified by NBS. The material is not certified for use after two years from date of shipment.

INFECTIOUS DISEASE TESTING

The supplier of this serum has tested the source materials used to prepare this product and found them to be nonreactive for Hepatitis B Surface Antigen (HB_sAg) and HIV by FDA approved testing. However, because no known test method can offer complete assurance that a human source material does not contain HIV, hepatitis, or other infectious agents these specimens should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 1984, 11-13.

ADDITIONAL CHARACTERIZATION OF SRM 1952 BY THE CENTERS FOR DISEASE CONTROL, ATLANTA, GA

Reference Method Values: Cholesterol was determined by Charlene Griffin using the modified Abell-Kendall Reference Method [3,4] developed by the Centers for Disease Control, Atlanta, GA. The results, as reported by CDC, are as follows:

Concentration Level	Mean Cholesterol Concentration, mg/dL	Standard Deviation	Number of Determinations	CV (%)
Low (1952-1)	210.50	1.24	12	0.59
Medium (1952-2)	267.17	1.70	12	0.64
High (1952-3)	362.25	1.60	12	0.44

REFERENCES:

1. Welch, M.J., Cohen, A., Ellerbe, T., Schaffer, R., Sniegoski, L.T., and White V, E., An isotope dilution capillary column GC/MS method for the accurate determination of serum cholesterol; Presented at the 35th ASMS Conference on Mass Spectrometry and Allied Topics, Denver, Colorado, May 24-29, (1987).

2. Cohen, A., Hertz, H.S., Mandel, J., Paule, R.C., Schaffer, R., Sniegoski, L.T., Sun, T., Welch, M.J., and White V, E., Total serum cholesterol by isotope dilution/mass spectrometry: A candidate definitive method. Clin. Chem., 26, 854-860, (1980).

3. Duncan, I.W., Mather, A., and Cooper, G.R. The procedure for the proposed cholesterol reference method. Clinical Chemistry Division, Center for Environmental Health, US Dept. of Health and Human Services, Public Health Service, Atlanta, GA, Centers for Disease Control, (1982).

4. Cooper, G.R. et al., The interlaboratory testing of the transferability of a candidate reference method for total cholesterol in serum, Clin. Chem., 32, 921-929, (1986).