ReCCS
(Reference Material Institute for Clinical Chemistry Standards)

Certificate of Analysis

Certified Reference Material for Measurement of Total Cholesterol in Human Serum

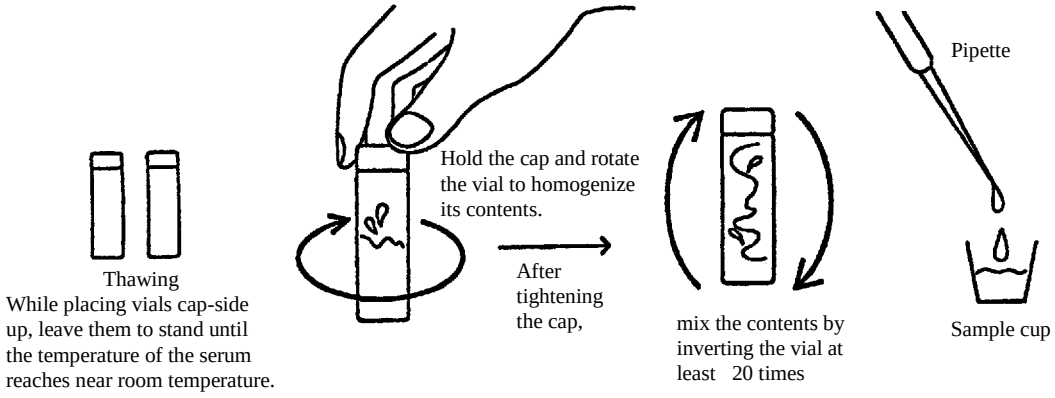
JCCRM 211-3

Intended use
JCCRM 211-3 is a certified reference material intended for use in evaluating the accuracy of total cholesterol assays as part of clinical laboratory tests and validating secondary or working reference materials.

Preparation
JCCRM 211-3 was prepared according to the protocol established by the National Committee for Clinical Laboratory Standards (NCCLS) [1] to ensure that, while avoiding lipoprotein degradation, its properties would be the same as those of fresh serum.

Product specifications
Configuration: Frozen liquid
Levels and contents: Two concentration levels: Medium concentration (white cap) and High concentration (black cap)
A single set of JCCRM 211-3 consists of four vials: two vials for each of the two concentration levels, and each vial contains 0.5 ml of human serum.

Use: IMPORTANT MATTERS
A vial is taken out from its case and is thawed at room temperature while placing it cap-side up. Next, the vial is left standing for about 30 minutes to bring the temperature of the serum to room temperature. Once this procedure is completed, hold the cap of the vial; gently rotate the vial in complete circles; and then mix the contents of the vial by inverting the vial up and down at least 20 times (the reference material in this manner must be used within two hours. Once thawed, the serum cannot be frozen for reuse.



Storage and expiration after purchasing
JCCRM 211-3 should be stored in a deep freezer upon receipt. When stored at a temperature below –70°C, its expiration date is 6 months from the date of shipping indicated below. When stored at a temperature from –40°C to –20°C, its expiration date is 2 weeks from the date of shipping indicated below.

Shipping date:

Precautions for use
JCCRM 211-3 is a human source material, and handle it as a biohazardous material capable of transmitting infectious disease. This product has been shown to be non-reactive for HBs antigen, HCV and HIV antibodies by our test methods. However, no known test method can give complete assurance of absence of HIV, HCV antibodies, HBs antigen, and any other infectious agents. Thus assume that JCCRM 211-3 would be infectious, and exercise the same caution as for handling any other clinical specimens with the risk of infectious diseases.

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JCCRM 211-3

Certified concentrations **Temperature = 25℃**

Levels	Total cholesterol concentrations and uncertainties
JCCRM 211-3M	**4.803 ± 0.018 mmol/L (185.7 ± 0.7 mg/dL)**
JCCRM 211-3H	**6.259 ± 0.021 mmol/L (242.0 ± 0.8 mg/dL)**
JCCRM224-5	**59.3±1.2mg/dL**

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The above total cholesterol concentrations were determined by isotope dilution-mass spectrometry [2, 3].
The analytical measurements were performed at the Laboratory of ReCCS by K. Nishiura and W. Tani (Technical Chief).
The expanded uncertainty U (95% confidence interval) shown in the above table for each certified value is obtained from the equation U=ku, where u is the combined standard uncertainty calculated according to the ISO Guide 35 [7] and k is a coverage factor. Total cholesterol was measured five times in one day on 15 separate days. Then the coverage factor, the Student's t distribution with 14 degrees of freedom, was k=2.145. Tractability to SI units was assured though calibration against NMIJ primary standards (purity 99.9 ± 0.1%) and other pertinent standards traceable to SI units as well as observance of ISO 17025 quality assurance. Standard solutions and sample solutions used in the measurements were prepared by gravimetric method using a calibrated balance.

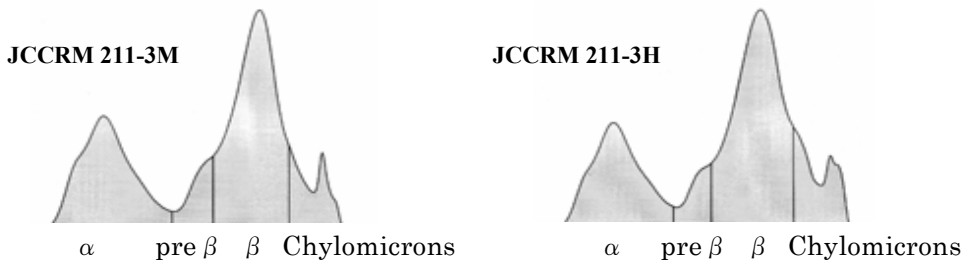
Characteristics
The characteristics of JCCRM 211-3 are as follows:

		JCCRM 211-3M	JCCRM 211-3H	
Density	(g/cm^3, 25°C)	1.0220	1.0235	Pycnometer method
Total protein	(g/dl)	6.8	7.2	Biuret method
Albumin	(g/dl)	4.2	4.5	BCG method
Triglycerides	(mg/dl)	117	153	Enzymatic method
Free fatty acids	(mEq/l)	0.43	0.46	Enzymatic method
Phospholipids	(mg/dl)	217	252	Enzymatic method
Cholesterol ester ratio	(%)	77	78	Enzymatic method
Lp(a)	(mg/dl)	8.0	5.8	Latex agglutination turbidimetric method
Uric Acid	(mg/dl)	5.3	5.8	Uricase-POD method
Total bilirubin	(mg/dl)	0.7	0.6	Vanadic acid oxidation method
Turbidity	Abs	0.2	0.2	Optical density method 710nm, 1cm

PAGE electrophoresis fraction ratios (%)

	211-3M	211-3H	Fresh serum
VLDL	9	12	9
MIDBAND	4	6	4
LDL	47	51	49
HDL	40	31	38

Agarose gel
electrophoresis pattern



Certification April 22, 2010

Masao Umemoto, Ph. D.

Head of Laboratory of ReCCS (Reference Material Institute for Clinical Chemistry Standards)

Reference data for total cholesterol

1. Comparison of total cholesterol values measured by the CDC reference method and ID/MS method
The total cholesterol concentrations measured by the CDC reference methods were higher for both levels. The reason for this is that the color fixing agent (Liebermann-Burchard reagent) used in the CDC reference method reacts with compounds other than cholesterol.

Table 1: Total cholesterol concentrations measured by two different methods

	ID/MS (mg/dL)	CDC Reference Method* (mg/dL)	Bias of the CDC Reference Method from the ID/MS Method
JCCRM 211-3M	185.7	187	+ 0.7 %
JCCRM 211-1H	242.0	243	+ 0.4 %

*: Measured at the Laboratory of ReCCS [4]

2. Evaluation of the validity of the ID/MS technique
JCCRM 211-3 reference materials were measured parallel with the Standard Reference Material NIST SRM 1951b (Level I, Level II) at the Laboratory of ReCCS by ID/MS technique, and the measured values for both NIST SRM 1951b Level I and Level II coincided 0.2% within the certified values (Table 2):

Table 2: NIST SRM 1951b total cholesterol concentrations measured by ID/MS (mg/dL)

Level	NIST certified concentrations and uncertainties	Measured concentrations*	Measured concentrations/ NIST certified concentrations Ratio
Level I	185.76 ± 0.55	186.0	1.0013
Level II	266.58 ± 0.84	266.3	0.9989

*: Measured at the Laboratory of ReCCS by ID/MS technique (average, n = 18)

3. Comparison of ID/MS techniques among CCQM laboratories
The National Institute of Materials and Chemical Research (NIMC), Japan, participated in the international comparison of total serum cholesterol concentrations hosted by the International Committee for Weights and Measures (Comité International des Poids et Mesures: CIPM) and the Consultative Committee for Amount of Substance (CCQM) (Comité Consultatif pour la Quantité de Matière). In the 2000 year comparison, the Laboratory of ReCCS performed ID/MS together with NIMC to measure total cholesterol concentrations in the CCQM samples. The following figure shows the results.

The results were favorable (2.181-2.217 mg/g) in all laboratories except for the NMI. In other words, the mean concentrations were within ±1% of the overall mean concentration at the five facilities, excluding the NMI. The mean concentration obtained at the Laboratory of ReCCS was within ±0.5% of the overall mean concentration.

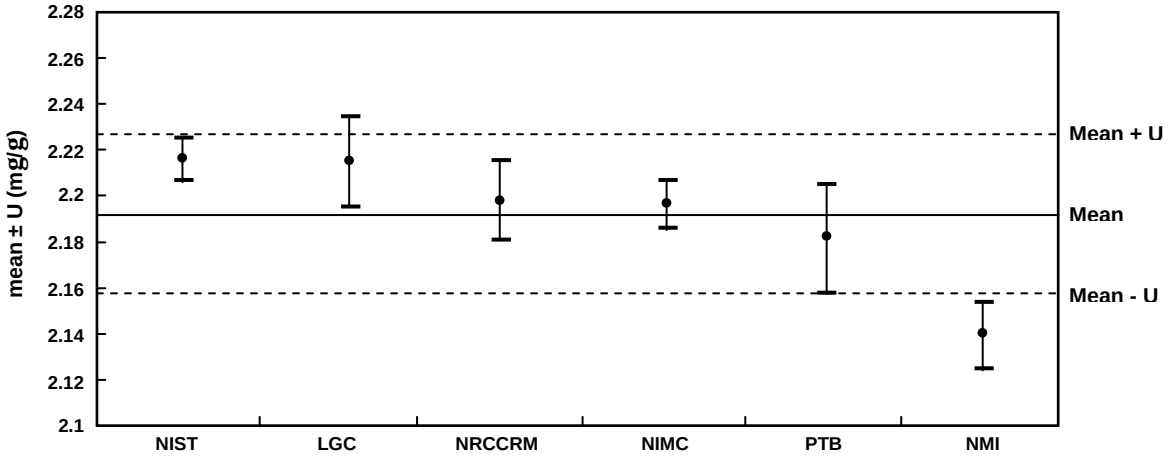


Figure 1: Comparison of total cholesterol concentrations measured at six CCQM laboratories

REFERENCES

[1] "Preparation and Validation of Commutable Frozen Serum Pools as Secondary Reference Materials for Cholesterol Measurement Procedures; Approved Guideline," NCCLS Publication C37-A, National Committee for Clinical Laboratory Standard, Wayne, PA, (1999).

[2] Cohen A., et al.: Total Serum Cholesterol by Isotope Dilution/Mass Spectrometry; A Candidate Definitive Method, Clin. Chem. 26 (1980), pp. 854-860.

[3] Ellerbe, P., Meiselman, S., Sniegoski, L.T., Welch, M.J., and White, V.E., "Determination of Serum Cholesterol by a Modification of the Isotope Dilution Mass Spectrometric Definitive Method," Anal. Chem. 61 (1989), pp. 1718-1723.

[4] Abell, L.L., Levy, B.B., Brodie, R.B., and Kendall, F.E., "Simplified Method for the Estimation of Total Cholesterol in Serum and Demonstration of Its Specificity," J. Biol. Chem. 195 (1952), pp. 357-360.

[5] Cooper, G.R., Smith, S.J., Duncan, I.W., et al., "The Interlaboratory Testing of the Transfer Ability of a Candidate Reference Method for Total Cholesterol in Serum," Clin. Chem. 32 (1986), pp. 921-929.

[6] *Guide to the Expression of Uncertainty in Measurement*, ISBN 92-67-10188-9, 1st Ed., ISO. Geneva, Switzerland (corrected and reprinted, 1995).

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