

# Reference Material Institute for Clinical Chemistry Standards (ReCCS)

Certified Reference Material for Measurement of  
Total Cholesterol and Glycerides in Human Serum



## JCCRM 211-8

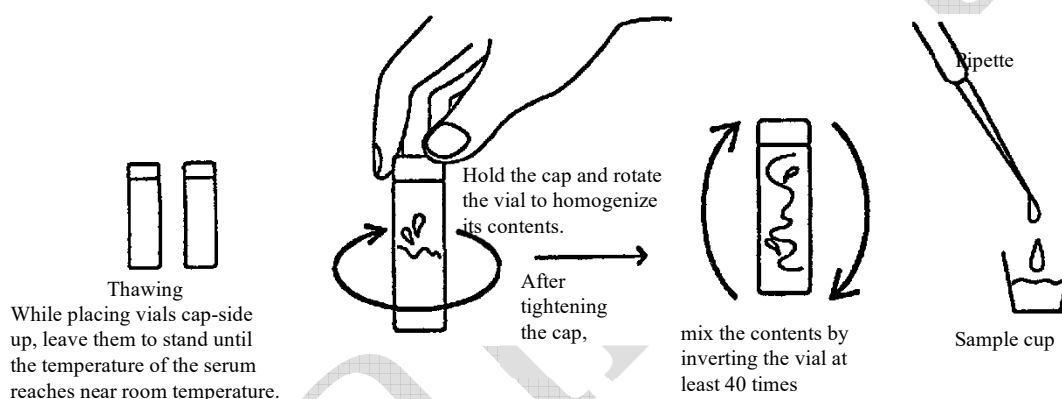
### Certificate of Analysis

#### ■ Intended use

This Certified Reference Material (CRM) is intended for use in evaluating the accuracy of Total Cholesterol, Total Glycerides and Triglycerides measurements in Laboratory Medicine, and a validating secondary reference material.

#### ■ Instruction for use

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 40 times in complete circles; and then mix the contents of the vial by turning the vial upside down at least 20 times (the reference material in this manner must be used within three hours. Once thawed, the serum cannot be frozen for reuse.



#### ■ Precautions for use : *In Vitro Use Only*

This CRM has been shown to be non-reactive for HBs antigens, HCV and HIV antibodies. However, no known test method can give complete assurance of absence of HIV, HCV, HBs and antigens, and any other infectious agents. Thus assume that this CRM would be infectious, and exercise the same caution as for handling any other clinical specimens with the risk of infectious diseases.

#### ■ Preparation

This CRM was prepared according to the protocol established by the Clinical and Laboratory Standards Institute (CLSI)C-37A<sup>1)</sup> to ensure that, while avoiding lipoprotein degradation, its properties would be the same as those of fresh serum.

#### ■ Storage and expiration

This CRM must be stored in a deep freezer upon receipt.

- 70°C below : 6 months from shipping date
- 40°C : 3 months from shipping date

#### ■ Product specifications

A single set of JCCRM 211-8 consists of 3 vials indicated below, each vial contains 0.5ml of human serum.

- JCCRM 211-8 (TC/M) (TG/M, FG)
- JCCRM 211-8 (TC/H)
- JCCRM 211-8 (TG/H,FG)

#### ■ Date of Certification

May 18, 2023

### ■ Certified Concentration Values of Total Cholesterol

The Total Cholesterol concentrations and uncertainties at 25°C below were measured by isotope dilution-mass spectrometry, ID/MS [3,4]. Since the analytical measurements were performed at the Laboratory of ReCCS as well as observance of ISO 17025 and ISO15195 quality assurance, these values are accepted internationally via ILAC/APLAC MRA.



Levels	Total Cholesterol
JCCRM 211-8(TC/M)	4.384 ± 0.047 mmol/L (169.5 ± 1.8 mg/dL)
JCCRM 211-8(TC/H)	5.085 ± 0.055 mmol/L (196.6 ± 2.1 mg/dL)

The expanded uncertainty  $U$  (95% confidence interval) shown in the above table is obtained by  $U=ku_c$ .  $u_c$  is the combined standard uncertainty calculated according to the ISO GUM<sup>2)</sup> (uncertainty of SRM and uncertainty of gravimetric method are also included) and  $k$  is a coverage factor ( $k = 2$ ).

### ■ Certified Concentration values of Total Glycerides and Free Glycerol

The Total Glycerides and Free Glycerol concentrations and uncertainties at 25°C below were measured by ID/MS<sup>3,4,5)</sup>. Triglycerides (TG) are defined as TG = Total Glycerides - Free Glycerol.<sup>6,7)</sup>

	Total Glycerides	Free Glycerol
JCCRM 211-8 (TG/M, FG)	1.355 ± 0.026 mmol/L (120.0 ± 2.3 mg/dL)	0.037 ± 0.001 mmol/L (3.3 ± 0.1 mg/dL)
JCCRM 211-8 (TG/H, FG)	2.017 ± 0.036 mmol/L (178.6 ± 3.2 mg/dL)	0.067 ± 0.002 mmol/L (5.9 ± 0.2 mg/dL)

The expanded uncertainty  $U$  (95% confidence interval) shown in the above table is obtained by  $U=ku_c$ .  $u_c$  is the combined standard uncertainty calculated according to the ISO GUM<sup>2)</sup> (uncertainty of SRM and uncertainty of gravimetric method are also included) and  $k$  is a coverage factor ( $k = 2$ ).

### ■ Traceability

Traceability to SI units was assured via calibration of Total cholesterol was conducted using NIST Standard Reference Material 911c (purity 99.2 ± 0.4%) as a calibrator.

Traceability to SI units was assured via calibration of Total Glycerides and Free Glycerol was conducted using NIST Standard Reference Material 1595 (purity 99.5 ± 0.2%) as a calibrator.

NIST SRM 1951c was measured at the same measurement run to check the accuracy of the measurements for Total Cholesterol.

## Supplemental Data JCCRM 211-8

### ■ Triglycerides concentrations by the ID/MS

Table 1. Triglycerides concentrations and uncertainties by the ID/MS

	Triglycerides(mg/dL)
JCCRM 211-8 (TG/M,FG)	116.7 ± 2.3
JCCRM 211-8 (TG/H, FG)	172.7 ± 3.2

Uncertainty is expressed as expanded uncertainty,  $U$ , calculated as  $U=kU$ , where  $U$  is the combined standard uncertainty calculated according to the ISO Guide <sup>2)</sup> where homogeneity, stability and others, etc. are all combined, and  $k$  is a coverage factor. The

coverage factors (95 % level of confidence) are all  $k=2$ . The triglycerides concentration equals the total concentration of triglycerides, diglycerides and monoglycerides, where free glycerol is not included in triglycerides.

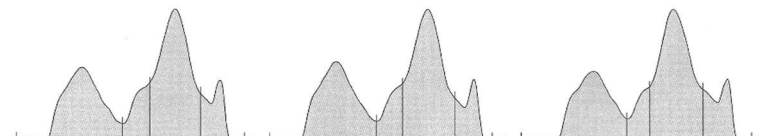
### ■ IFCC RELA Proficiency Test

ReCCS demonstrates goods results in 2018 (Total Cholesterol) and 2017(Total Glycerides) RELA (IFCC External Quality assessment scheme for Reference Laboratories in Laboratory Medicine) Proficiency test .<sup>8)</sup>

### ■ Characteristics of This CRM

Table 2. The characteristics of JCCRM 211-8

		TC/M	TC/H	TG/H, FG	
Total protein	(g/dL)	6.9	6.9	7.0	Biuret method
Albumin	(g/dL)	4.1	4.2	4.2	BCG method
Lp(a)	(mg/dL)	16.1	17.0	3.9	Latex agglutination
Uric Acid	(mg/dL)	5.4	5.2	5.0	Enzymatic method
Total bilirubin	(mg/dL)	0.5	0.4	0.4	Vanadic acid oxidation method
Density	(g/cm <sup>3</sup> )	1.024	1.024	1.024	Pycnometer method(25°C)



	TC/M	TC/H	TG/H, FG
① α -LP	32.1 %	34.3 %	30.1 %
② pre β -LP	10.8 %	10.3 %	8.6 %
③ β -LP	46.1 %	45.5 %	47.9 %
④ chylomicron	11.0 %	9.9 %	13.4 %

Fig 1. Agarose gel electrophoresis pattern

### ■ References

- 1) CLSI Publication C37-A. Preparation and validation of commutable frozen human serum pools as secondary reference materials for cholesterol measurement procedures; approved guideline: CLSI, Wayne, PA, 1999.
- 2) Evaluation of measurement data - Guide to the expression of uncertainty in measurement. ISO/IEC Guide 98-3 (JCGM 100:2008).
- 3) Cohen A. et al. Total serum cholesterol by isotope dilution/mass spectrometry; A candidate definitive method, Clin Chem 1980;26:854-860.
- 4) Ellerbe P, Meiselman S, Sniegoski LT, Welch MJ, White VE. Determination of serum cholesterol by a modification of the isotope dilution mass spectrometric definitive method. Anal Chem,1989;61:1718-1723.
- 5) Edwards SH, Kimberly MM, Pyatt SD, et al. Proposed serum cholesterol reference measurement procedure by gas chromatography-isotope dilution mass spectrometry. Clin Chem 2011;57:614-622.
- 6) Ellerbe P, Sniegoski LT, Welch MJ. Isotope dilution mass spectrometry as a candidate definitive method for determining total glycerides and triglycerides in serum. Clin Chem 1995;41:397-404.
- 7) Bernert JT Jr, Bell CJ, McGuffey JE, Waymack PP. Determination of "free" glycerol in human serum reference materials by isotope-dilution gas chromatography-mass spectrometry. J Chromatogr 1992;578:1-7.
- 8) RELA - IFCC External Quality assessment scheme for Reference Laboratories in Laboratory Medicine( <http://www.dgkl-rfb.de:81/>)

### Provider of JCCRM 211-8

*Hirohito Umemoto Ph.D.*

Hirohito Umemoto, Ph.D. (President)

Certificate Revision		
R0	2020.9.8	Original certificate issue date
R1	2021.7.15	Contact modification
R2	2023.5.18	Uncertainty change

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