Reference Material Institute for Clinical Chemistry Standards (ReCCS)

Certified Reference Material for Measurement of HDL-cholesterol, LDL-cholesterol and Triglycerides in Human Serum

JCCRM 224-19

Certificate of Analysis

ASNITE 0006 R

This Certified Reference Material (CRM) was manufactured and certified by ReCCS according to ISO Guide 34 (general requirements regarding the ability of reference material producers), ISO/IEC17025 (requirements of calibration laboratory) and ISO15195 (requirements of clinical laboratory standard measurement institutes).

■ Intended use

This CRM is intended primarily for use in evaluating the accuracy of clinical procedures for the determination of HDL-cholesterol(HDL-C), LDL-cholesterol(LDL-C) and triglycerides.

■ Certified concentration values and uncertainties

Metrological traceability is to the SI derived unit for mass concentration (expressed as milligram per deciliter). Since these values are certified according to ISO/IEC17025 and ISO15195, these are accepted internationally via ILAC/APL AC MRA (exclude the value for Triglycerides)

ASNITE 0006 C

Table 1. Certified Mass Concentration Values for HDL-C and LDL-C

Unit: mg/dL

Item	HDL-C (CDC reference Method)		LDL-C (CDC reference Method)	
	Certified value	Uncertainty	Certified value	Uncertainty
(I)	48.1	1.6	—	
(II)	—		99.7	1.9

Table 2. Certified Mass Concentration Value for Triglycerides

Unit: mg/dL

Item	Triglycerides (ID/GC-MS)	
	Certified value	Uncertainty
(III)	116.7	2.3

The expanded uncertainty U (95 % level of confidence) shown for each certified value in the above table is obtained from the equation $U=ku$, where u is the combined standard uncertainty (the purity of triolein was also combined) calculated according to the ISO Guide[2)], and k is a coverage factor. The coverage factor k, determined from the Student's t distribution, is k=2.0.

The concentration of the above triglycerides was calculated by equation [total glycerides－free glycerol] where total glycerides and free glycerol were assayed by ID/GC/MS.

■ Measurement methods for certified values

HDL-C: The CDC reference methods and ID/GC-MS [3), 4), 5), 6), 7)]. The measured values were calculated to adjust to Abell-Kendall method.
LDL-C: CDC beta-quantification reference method for LDL-C in serum and ID/GC-MS [3), 4), 5), 8)]. The measured values were calculated to adjust to Abell-Kendall method.
The concentrations of HDL-C and LDL-C were assayed by three international reference laboratories (two are CDC CRMLN laboratories and the other is Laboratory of ReCCS).
Triglycerides: ID/GC-MS [9), 10)].

■ Instructions for use

Remove a glass vial containing this CRM from the bag and stand it by placing the vial upright. Leave the vial to stand for about 30 minutes to bring the temperature of the content to room temperature. Hold the vial upright by the cap and then mix the content by turning the vial upside down at least 40 times. This CRM should be used within 3 hours and the rest of the material in the vial should not be refrozen for afterward use.

■ Precautions for use *in vitro* use only

This CRM was prepared from human serum and shown to be negative to the HBs antigens, HCV antibodies and HIV antibodies. However, this does not completely rule out its infectivity, and when handling this CRM, exercise the same caution used for any other patient specimens: Recommended to be handled at the biosafety Level 2.

■ Storage and expiration

This CRM is shipped in frozen condition on dry ice. Confirm that dry ice remains upon receipt, otherwise the materials cannot be used thereafter.
Store this CRM in a freezer immediately after receiving it.
The expiration date is as follows from the shipping (see the label of the outer case).

HDL-C & LDL-C HDL-C	−70 ℃:	1 month	−40 ℃:	1 week
Total cholesterol & Triglycerides (Total glycerides)	−70 ℃:	6 month	−40 ℃:	3 month

■ Specification

JCCRM 224-19 (I) (II) (III) levels, 0.5 ml/vial for each level, 3 vials in total.

■ Preparation

This CRM was prepared from pooled human serum as fresh as possible according to CLSI (Clinical and Laboratory Standards Institute) C37-A [1)] document “Preparation and validation of commutable frozen serum pools as secondary reference materials for cholesterol measurement procedures”, of which specifications are at least satisfied with this CRM and its commutability is verified [2)].

■ Traceability

The ID/GC-MS for HDL-C and LDL-C was conducted using NMIJ CRM 6001-a (purity 99.9±0.1 %) as a calibrator.
The ID/GC-MS for triglycerides was conducted using NIST SRM 1595 (purity 99.5 %±0.2 %) as a calibrator.

■ Date of Certification

September 30 , 2022

Reference

■ Characteristics

The characteristics of this CRM are tabulated below:

Item	JCCRM 224-19			unit	Method
	(I)	(II)	(III)		
Total Protein	7.2	7.4	7.1	g/dL	Biurette method
Albumin	4.1	4.2	4.3	g/dL	BCG colorimetric method
Lp(a)	9.5	12.0	23.1	mg/dL	LA
apo E	3.0	2.8	3.5	mg/dL	TIA
VLDL	21	–	21	%	PAGE
IDL	–	–	6	%	
LDL	41	–	39	%	
HDL	35	–	33	%	
Uric acid	4.6	5.3	7.1	mg/dL	Enzymatic method
Total Bilirubin	0.4	0.3	0.4	mg/dL	Vanadic acid oxidation method
Hemoglobin	Less than 4	Less than 4	Less than 4	mg/dL	Colorimetry

■ 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) N Rifai, GR Warnick, MH Daminiczak. Handbook of lipoprotein testing, 2nd Ed. pp.227-230, AACC Press, 2000, Washington DC.
7) Warnick GR, Albers JJ. Heparin-Mn2+ quantitation of high-density-lipoprotein cholesterol: An ultrafiltration procedure for lipemic samples. Clin Chem 1978; 24:900-904.
8) Bachorik PS, Ross JW. National Cholesterol Education Program recommendations for measurement of low-density lipoprotein cholesterol. Clin Chem 1995; 41:1414-1420.
9) 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.
10) 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.

Provider of JCCRM 224-19

Hirohito Umemoto Ph. D.

Hirohito Umemoto, Ph.D. (President)

Reference Material Institute for Clinical Chemistry Standards (ReCCS)
1050-35, Ichigaocho, Aoba-ku, Yokohama 225-0024
Telephone: 81-45-507-6145
Facsimile: 81-45-530-9036
E-mail: overseas@reccs.net
URL:http://www.reccs.or.jp