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Certificate of Analysis

CERTIFIED REFERENCE MATERIAL
HRM-3008A

Total Cholesterol, HDL-Cholesterol, LDL-Cholesterol, and Total Glycerides in Frozen Human Serum

Batch Number
STY-0018-099
STY-0018-100

Foreword
A unit of the certified reference material (CRM) HRM-3008A consists of two vials of frozen human serum with different analyte concentration levels. Each vial contains 1 mL of frozen human serum. Each serum is certified for four analytes (total cholesterol, HDL-cholesterol, LDL-cholesterol, and total glycerides) and is stored in glass vials with crimp-cap. The serum materials appear as a transparent (or slightly cloudy) brownish yellow liquid after thawing.

The CRM was produced with reference to the requirements set out in ISO/IEC 17025:2017 [1], ISO 17034:2016 [2] and ISO Guide 35:2017 [3].

Certified Concentration Values
The certified concentration values for all analytes in HRM-3008A are provided in Table 1. The amount-of-substance concentration values for all analytes, except total glycerides*, were calculated from mass concentration values (expressed per mass), the measured serum densities at about 25 ^{o}C (1.02250 ± 0.00031 g/mL and 1.02253 ± 0.00033 g/mL for STY-0018-099 and STY-0018-100, respectively) and the relative molecular masses of the analytes [386.65 (total cholesterol, HDL-

* Total glycerides in this CRM contain a mixture of triglycerides, diglycerides, monoglycerides and free glycerol.

cholesterol, LDL-cholesterol)]. The amount-of-substance concentration values (expressed per volume) of total glycerides were converted from amount-of-substance concentration values (expressed per mass) and the respective measured serum densities only.

Table 1. Certified Concentration Values of Analytes in HRM-3008A

Analyte	STY-0018-099 (Level 1) (mmol/L)	STY-0018-100 (Level 2) (mmol/L)
Total Cholesterol	4.75 ± 0.11	5.47 ± 0.11
HDL-Cholesterol	1.323 ± 0.027	1.269 ± 0.025
LDL-Cholesterol	2.819 ± 0.090	3.402 ± 0.093
Total Glycerides	1.540 ± 0.037	1.914 ± 0.046

Each certified concentration value is expressed as the certified concentration value ± the expanded uncertainty.

Each certified concentration value is the mean of measurements of six samples taken from three bottles. The certified concentrations for HRM-3008A were determined using the isotope dilution mass spectrometric (IDMS) method. A four-point calibration curve was used in the measurements.

The associated measurement uncertainty of each certified concentration value was evaluated in accordance with ISO/IEC Guide 98-3:2008 [4]. The expanded uncertainty (coverage factor of 2) corresponded to a level of confidence of about 95%.

Validity

The certified concentration values of HRM-3008A are valid within the specified measurement uncertainty until **15 Apr 2029**. The validity of HRM-3008A will be extended if it is tested to be sufficiently stable for continuous use. The certified concentration values of HRM-3008A are invalid when the serum material has deteriorated or is mishandled.

Source of Materials

The serum materials were prepared by Solomon Park Research Laboratories (Kirkland, WA, USA) from normal human serum, following National Committee for Clinical Laboratory Standards (NCCLS) C-37A guidelines [5]. The collection of blood, isolation of serum, pooling of individual liquid serum units, mixing, aliquoting and freezing of pools were carried out sequentially within 56 hours of the blood collection.

Commutability

Commutability study of HRM-3008A was conducted by analysing 30 patient serum samples and the CRM by both IDMS method and the routine methods using various analysers, which included Roche Cobas c702, Roche Cobas c311, Siemens Atellica CH, Beckman Coulter AU5800, and Abbott Architect c16000. The commutability of HRM-3008A for different analytes and different analysers were evaluated using both linear regression model suggested by the standard CLSI EP30-A (formerly CLSI C53-A) [6] and the log-transformed model recommended by IFCC [7,8]. The CRMs were found to be commutable for all analytes on all five analysers using CLSI model or IFCC model for evaluation except poor commutability for LDL-cholesterol were observed on Siemens Atellica CH. This suggested that although the commutability of HRM-3008A is generally satisfactory for most of the clinical analysers, it may not be satisfactory for all clinical analysers. For some individual clinical

analysers, such as Siemens Atellica CH, there may be some deviations between the results from routine testing methods and the certified values of HRM-3008A for some of the analytes, such as LDL-cholesterol.

Homogeneity

Based on ISO 17034:2016 [2], knowledge on the homogeneity of total cholesterol and total glycerides in HRM-3008A was drawn from prior evidence on two previous batches of close similar serum CRMs (HRM-3002B). The serum materials of HRM-3002B and HRM-3008A were prepared by the same laboratory following the same guideline [6]. The homogeneity testing of HRM-3002B was conducted on two sub-samples taken from at least ten bottles. A Roche Cobas C501 chemistry analyzer was used for the homogeneity testing. The sample size taken for homogeneity testing was in the range of 2 to 3 µL. No significant differences in the between and within-bottle variances were found using *F*-test (ANOVA) at 95 % confidence level. The u_{bb} was evaluated from the uncertainty due to between-bottle inhomogeneity.

Stability

Based on ISO 17034:2016 [2], knowledge on the stability of total cholesterol and total glycerides in HRM-3008A stored at a temperature of below – 60 °C was drawn from previous experience on HRM-3002B. The stability testing of HRM-3002B was conducted on at least three occasions over a period of up to five months using IDMS method. The stability of HRM-3002B was also monitored using IDMS method for up to 6 years. The results showed that the analytes in such frozen serum CRMs were stable when stored at below – 60 °C over the study period [3]. The u_{stab} was evaluated from the standard error of the slope.

Analytical Methods

For the determination of total cholesterol, a fully validated gas chromatography-isotope dilution mass spectrometry (GC-IDMS) method was used [9]. The method involved spiking with isotope labeled cholesterol, hydrolysis in potassium hydroxide solution, extraction into cyclohexane, derivatisation using N,O-bis(trimethylsilyl)acetamide, separation using a DB5-MS column, followed by mass spectrometric measurement.

For the determination of total glycerides, a fully validated GC-IDMS method was used [10,11]. The method involved spiking with isotope labeled tripalmitin, hydrolysis in ethanolic potassium hydroxide solution, extraction using mixed mode cation exchange SPE 60 mg / 3 mL cartridge, two-step derivatisation using 1-butylboronic acid and N-methyl-N-trimethylsilylfluoroacetamide, separation using a DB5-MS column, followed by mass spectrometric measurement.

For the determination of HDL-cholesterol and LDL cholesterol, a fully validated beta-quantification method [12] followed by cholesterol determination using GC-IDMS method was used. The method involved the separation of the bottom fraction (including both HDL-cholesterol and LDL-cholesterol) from the serum and precipitation of LDL-cholesterol, followed by the application of the same GC-IDMS method used in the measurement of cholesterol as described above.

Metrological Traceability

The certified concentration values are traceable to the International System of Units (SI) through the use of cholesterol and tripalmitin CRMs from NIST (SRM 911c and SRM 1595, respectively).

Intended Use

HRM-3008A is a secondary reference material intended for use in the validation of methods or as quality control materials for the determination of total cholesterol, HDL-cholesterol, LDL-cholesterol, and total glycerides in human serum. Users may refer to ISO 33403:2024 [13] for the recommended

statistical treatment of the certified reference value and the associated uncertainty of the CRM as control materials.

Warning and Safety Precautions for Users

HRM-3008A is intended for in-vitro use only and shall be handled as a biohazardous material with the potential of transmitting infectious disease. Hence, this material shall be handled using biosafety level 2 (or higher) practices, equipment, and facility [14].

Instructions for Use

While the supplier has reported that each donor unit of serum used in the preparation of the serum materials has been tested by an FDA approved method and found to be non-reactive for HbsAg and HIV-1 antibody, no known test method can offer complete assurance that hepatitis B virus, HIV or other infectious agents are absent from the materials. Accordingly, these materials should be handled and disposed according to associated regional, national and local legislation and regulations for any potentially infectious human or blood specimen.

Prior to use, the HRM-3008A should be thawed at room temperature (between 18 ^{o}C to 25 ^{o}C), then analysed immediately. The materials should be mixed well by gentle swirling before withdrawing any aliquots. The certified concentration values may not be valid for re-thawed and opened bottles as the stability of all analytes subjected to such conditions has not been investigated.

The minimum sample size should be 3 µL. The certified values may not be valid if smaller amounts are taken.

Transport and Storage

HRM-3008A is transported in frozen state (in dry ice). Upon receipt, it should be stored at below – 60 ^{o}C. HRM-3008A should not be exposed to sunlight or ultraviolet radiation. Storage of the thawed material at room temperature or in the refrigerator may result in changes in the concentrations of the analytes.

Further Information

Please direct all enquiries regarding this CRM to the contact in this COA.

References

[1] ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories.
[2] ISO 17034:2016 General requirements for the competence of reference material producers.
[3] ISO Guide 35:2017 Reference materials – Guidance for characterisation and assessment for homogeneity and stability.
[4] ISO/IEC Guide 98-3:2008 Uncertainty of measurement – Part 3: Guide to the expression of uncertainty in measurement (GUM: 1995).
[5] NCCLS Publication C37-A, National Committee for Clinical Laboratory Standards: Wayne, PA (2000).
[6] Clinical and Laboratory Standards Institute. EP30-A characterization and qualification of commutable reference materials for laboratory medicine; approved guideline. May 2010.
[7] Miller WG, Schimmel H, Rej R, Greenberg N, Ceriotti F, Burns C, et al. IFCC Working Group recommendations for assessing commutability. Part 1: general experimental design. Clin Chem, 2018, 64, 447-454.
[8] Nilsson G, Budd JR, Greenberg N, Delatour V, Rej R, Panteghini M, et al. IFCC Working Group recommendations for assessing commutability. Part 2: using the difference in bias between a reference material and clinical samples. Clin Chem, 2018, 64, 455-464.

[9] 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.
[10] 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, 41/3, 397-404.
[11] Chen Y, Liu Q, Yong S, Teo HL, Lee TK. An improved reference measurement procedure for triglycerides and total glycerides in human serum by isotope dilution gas chromatography–mass spectrometry. Clin Chim Acta, 2014, 428, 20-25.
[12] Myers GL, Cooper GR, Greenberg N, Kimberly MM, Waymack PP, Hassemer DJ. Standardization of Lipid and Lipoprotein Measurements; In Handbook of Lipoprotein Testing, 2nd ed. Rifai N, Warnick GR, Dominiczak MH, Eds. American Association for Clinical Chemistry (AACC), Washington, D.C., 2000, pp. 717-748.
[13] ISO Guide 33403:2024 Reference materials – Requirements and recommendations for use.
[14] U.S Department of Health and Human Services; Biosafety in Microbiological and Biomedical Laboratories, 5th ed.; HHS Publication No. (CDC) 21-1112.

Certificate Revision Records

Certificate Ref. No.	Date of issue	Reason for issuance
CML-HRM-3007A/01	19 May 2022	Issuance of first certificate
CML-HRM-3010A/02	15 Apr 2025	Extension of expiry date

Note

HSA does not assume any liability with respect to any loss caused by improper use and/or storage of the CRM by the customer.

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