

Certificate of Analysis

CERTIFIED REFERENCE MATERIAL HRM-3007A

Creatinine, Glucose, Urea and Uric Acid in Frozen Human Serum

Batch Number

STY-0018-097
STY-0018-098

Foreword

A unit of the certified reference material (CRM) HRM-3007A 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 (creatinine, glucose, urea and uric acid) 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-3007A are provided in Table 1. The amount-of-substance concentration values for all analytes were calculated from mass concentration values (expressed per mass), the measured serum densities at about 25 °C (1.02227 ± 0.00033 g/mL and 1.02230 ± 0.00031 g/mL for STY-0018-097 and STY-0018-098, respectively) and the relative molecular masses of the analytes [113.12 (creatinine), 180.16 (glucose), 60.06 (urea) and 168.11 (uric acid)].

Table 1. Certified Concentration Values of Analytes in HRM-3007A

Analyte	STY-0018-097	STY-0018-098
	(Level 1)	(Level 2)
	(mmol/L)	(mmol/L)
Creatinine	0.0954 ± 0.0028	0.2372 ± 0.0060
Glucose	5.38 ± 0.11	6.11 ± 0.13
Urea	6.14 ± 0.14	11.02 ± 0.28
Uric Acid	0.3234 ± 0.0082	0.500 ± 0.013

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-3007A were determined using 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-3007A are valid within the specified measurement uncertainty until **15 Apr 2029**. The validity of HRM-3007A will be extended if it is tested to be sufficiently stable for continuous use. The certified concentration values of HRM-3007A 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-3007A was conducted by analysing 28 to 30 patient serum samples, 27 to 30 patient plasma 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-3007A for different analytes, different patient samples (serum or plasma), 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 generally commutable for all analytes on all five analysers using CLSI model or IFCC model for evaluation. However, Level 1 of HRM-3007A was found to have poor commutability to patient serum samples for glucose on Siemens Atellica CH and uric acid on Roche Cobas c311; while Level 2 of HRM-3007A was found to have poor commutability to patient plasma samples for urea on Siemens Atellica CH, and to both patient serum samples and patient plasma samples for uric acid on Roche Cobas c311. This suggested that although the commutability of HRM-3007A 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 Roche Cobas c311, there may be some deviations between the

results from routine testing methods and the certified values of HRM-3007A for some of the analytes, such as uric acid.

Homogeneity

Based on ISO 17034:2016 [2], knowledge on the homogeneity of creatinine, glucose, urea and uric acid in HRM-3007A was drawn from prior evidence on two previous batches of closely similar serum CRMs (HRM-3002B). The serum materials of HRM-3002B and HRM-3007A 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. Liquid chromatography-isotope dilution tandem mass spectrometry (LC-IDMS/MS) method was used for the homogeneity testing of creatinine and urea. Gas chromatography-isotope dilution mass spectrometry (GC-IDMS) method was used for the homogeneity testing of glucose. A Roche Cobas C501 chemistry analyzer was used for the homogeneity testing of uric acid. The sample size taken for homogeneity testing was about 0.1 mL for IDMS method, and in the range of 2 to 3 μ L for chemistry analyzer method. 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 creatinine, glucose, urea and uric acid in HRM-3007A 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 creatinine, a fully validated LC-IDMS/MS method was used [9,10]. The method involved spiking with isotope labeled creatinine, precipitation of protein, separation using an Agilent Zorbax SB-Aq column, followed by tandem mass spectrometric measurement.

For the determination of glucose, a fully validated GC-IDMS method was used [11]. The method involved spiking with isotope labeled glucose, precipitation of protein, two-step derivatisation using hydroxylamine hydrochloride and acetic anhydride, separation using a DB5-MS column, followed by mass spectrometric measurement.

For the determination of urea, a fully validated LC-IDMS/MS method was used [12]. The method involved spiking with isotope labeled urea, precipitation of protein, separation using an Agilent Zorbax RX-SIL column, followed by tandem mass spectrometric measurement.

For the determination of uric acid, a fully validated LC-IDMS/MS method was used [13]. The method involved spiking with isotope labeled uric acid, precipitation of protein, separation using an Agilent Zorbax SB-Aq column, followed by tandem mass spectrometric measurement.

Metrological Traceability

The certified concentration values are traceable to the International System of Units (SI) through the use of creatinine, glucose, urea and uric acid CRMs from NIST (SRM 914b, SRM 917c, SRM 912b and SRM 913b, respectively).

Intended Use

HRM-3007A is a secondary reference material intended for use in the validation of methods or as quality control materials for the determination of creatinine, glucose, urea and uric acid in human serum. Users may refer to ISO 33403:2024 [14] 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-3007A 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 [15].

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, HRM-3007A should be thawed at room temperature (between 18 °C to 25 °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/or opened bottles as the stability of all analytes subjected to such conditions has not been investigated.

The minimum sample size should be 0.1 mL for creatinine, glucose and urea, and 3 µL for uric acid. The certified values may not be valid if smaller amounts are taken.

Transport and Storage

HRM-3007A is transported in frozen state (in dry ice). Upon receipt, it should be stored at below – 60 °C. HRM-3007A 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] Dodder NG, Tai S, Sniegowski LT, Zhang NF, Welch MJ. Certification of creatinine in a human serum reference material by GC-MS and LC-MS. Clin Chem, 2007, 53, 1694-1699.
- [10] Stokes P, O'Connor G. Development of a liquid chromatography-mass spectrometry method for the high-accuracy determination of creatinine in serum. J Chromatogr B, 2003, 794, 125-136.
- [11] Magni F, Paroni R, Bonini PA, Kienle MG. Determination of serum glucose by isotope dilution mass spectrometry: candidate definitive method. Clin Chem, 1992, 38/3, 381-385.
- [12] Yong S, Liu H, Wong L, Teo HL, Chen Y, Liu Q, et al. Liquid chromatography-isotope dilution tandem mass spectrometry method for the measurement of urea in human serum and assignment of reference values to external quality assessment samples. Int J Mass Spectrom, 2017, 414, 87-93.
- [13] Dai X, Fang X, Zhang C, Xu R, Xu B. Determination of serum uric acid using high-performance liquid chromatography (HPLC)/isotope dilution mass spectrometry (ID-MS) as a candidate reference method. J Chromatogr B, 2007, 857, 287-295.
- [14] ISO Guide 33403:2024 Reference materials – Requirements and recommendations for use.
- [15] 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.



Dr Teo Tang Lin
Division Director
Chemical Metrology Laboratory
Chemical Metrology Division