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Certificate of Analysis

CERTIFIED REFERENCE MATERIAL
HRM – 2005A

Calcium, Potassium, Sodium, Magnesium, Iron, and Chloride in Human Serum

Batch Number
STY-0018-020
STY-0018-021

Description
A unit of the certified reference material (CRM) HRM-2005A consists of two vials of frozen human serum of different analyte concentration levels. Each vial contains 1 mL of frozen human serum. Each serum contains six analytes (calcium, potassium, sodium, magnesium, iron, and chloride) and is stored in glass vial with crimp-cap. The serum material appears as a transparent (or slightly cloudy) brownish yellow liquid after thawing.

The reference material 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-2005A are provided in Tables 1 and 2. The certified concentration values for all analytes were calculated from mass fraction values, the measured serum densities at 21°C (1.0208 g/mL and 1.0267 g/mL for STY-0018-020 and STY-0018-021, respectively) and the relative molecular masses of the analytes [40.08 (calcium), 39.10 (potassium), 22.99 (sodium), 24.31 (magnesium), 35.45 (chloride), and 55.85 (iron)].

Table 1. Certified Concentration Values of Analytes

Analyte	STY-0018-020 (mmol/L)	STY-0018-021 (mmol/L)
Calcium	1.968 ± 0.053	2.484 ± 0.067
Potassium	3.625 ± 0.059	4.81 ± 0.15
Sodium	122.8 ± 2.3	163.9 ± 2.0
Magnesium	0.737 ± 0.031	0.960 ± 0.021
Chloride	92.0 ± 1.3	122.3 ± 4.3

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

Table 2. Certified Concentration Value of Analyte

Analyte	STY-0018-20 (µmol/L)	STY-0018-21 (µmol/L)
Iron	10.67 ± 0.45	14.13 ± 0.54

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

Each certified concentration value is the mean of measurements of at least six samples taken from a minimum of three bottles. The certified concentrations for calcium and sodium were determined by standard addition method using inductively coupled plasma-optical emission spectroscopy (ICP-OES). The certified concentration for potassium, magnesium, chloride, and iron were determined by isotope dilution mass spectrometry (IDMS) method using inductively coupled plasma-mass spectrometry (ICP-MS) or inductively coupled plasma high resolution-mass spectrometry (ICP-HR-MS).

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

Homogeneity

Homogeneity testing on HRM-2005A was performed on two sub-samples taken from at least ten bottles each by external calibration method using inductively coupled plasma mass spectrometry (ICP-MS) for all six analytes. The sample size taken for homogeneity testing was 0.1 g. No significant differences in the between and within-bottle variances were found using one-way ANOVA at 95 % confidence level [3]. Thus, the material was regarded to be sufficiently homogeneous. The u_{bb} was evaluated from the uncertainty due to between-bottle inhomogeneity.

Stability

Stability testing for HRM-2005A was performed at – 70 °C on at least three occasions over a period of up to six months. The slope of the fitted regression line was found to be insignificant at 95% confidence level [3]. Thus, the serum materials were regarded as sufficiently stable. The u_{stab} was evaluated from the standard error of the slope.

Validity of Certified Concentration Values

The certified concentration values of HRM-2005A are valid within the specified measurement uncertainties until **11 May 2027**. The validity of HRM-2005A will be extended if it is tested to be sufficiently stable for continuous use. The certified concentration values of HRM-2005A are invalid when the serum material has deteriorated or is mishandled.

Commutability

The methods used for the certification of HRM-2005A were all validated using the Standard Reference Material (SRM 956c) from NIST that is listed in the Joint Committee for Traceability in Laboratory Medicine (JCTLM) database.

In addition, all three frozen human sera of HRM-2005A were analysed by at least 15 clinical laboratories in Singapore in an External Quality Assessment (EQA) programme organised by HSA in 2014. All the clinical laboratories employed routine testing methods to determine the concentration of the analytes. The mean of the reported results were generally found to be comparable within the associated measurement uncertainty of the assigned values determined by HSA using the IDMS or the standard addition method. Commutability with patient samples has not been directly demonstrated. In the case where the material is used to calibrate an assay or for trueness check in validation, prior investigation of the commutability with patient samples for that assay must be carried out by the user. However, there was no evidence that it would be satisfactory for all the clinical analysers available commercially. For some analysers, there may be larger variations in the results of routine testing methods from the certified values of HRM-2005A.

Analytical Methods

For the determination of calcium and sodium, a multiple-point calibration standard addition method was used [5, 6]. The method involved digestion with 5% HNO_3 and spiking with different levels of calcium or sodium standard solutions, followed by ICP-OES measurement.

For the determination of potassium, magnesium and iron, an exact matching IDMS method was used [5]. The method involved spiking with isotope labeled standards, digestion with 5% HNO_3 or concentrated HNO_3, followed by ICP-HR-MS (potassium) or ICP-MS (magnesium and iron) measurement.

For the determination of chloride, an exact matching IDMS method was used [5]. The sample was spiked with isotope labeled standard. The proteins were removed using ammonium molybdate and chloride was precipitated using silver nitrate. The silver chloride was redissolved in concentrated ammonia, diluted with water, followed by ICP-HR-MS measurement.

Metrological Traceability

The certified concentration values are traceable to the International System of units (SI) through the use of calibration standards from the National Institute of Standards and Technology (NIST), USA (SRM 3109a for calcium, SRM 918b for potassium, SRM 919b for sodium and chloride, SRM 3131a for magnesium, and SRM 937 for iron).

Intended Use

HRM-2005A is intended for use in the validation of methods or as quality control materials for the determination of calcium, potassium, sodium, magnesium, iron and chloride in human serum. Users may refer to ISO Guide 33:2015 [7] for the recommended statistical treatment of the certified reference values and the associated uncertainties of the CRM as control materials.

Instruction for Use

While the supplier has reported that each donor unit of serum used in the preparation of the serum materials has been tested 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-2005A should be thawed at room temperature (between 18 ^oC to 25 ^oC), then analysed immediately. The materials should be mixed well by gentle swirling before withdrawing any aliquots. Users may apply the methods/procedures that they would normally apply to obtain the minimum sample size, provided that sufficient mixing is carried out. 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 0.1 g. The certified concentration values may not be valid if smaller amounts are taken.

Transport and Storage

The CRM is transported in frozen state (in dry ice). Upon receipt, it should be stored at below – 60 ^oC. HRM-2005A 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.

Safety Precautions for Users

HRM-2005A 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 [8].

Further Information

Please direct all enquiries regarding this CRM to the contact in this Certificate of Analysis.

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] Long, S. E.; Murphy, K. E.; NIST Special Publication 260-162, Government Printing Office: Washinton, DC (2006).
[6] Simpson, L. A.; Hearn. R.; Merson, S.; Catterick, T.; Talanta, 2005, 65, 900-906.
[7] ISO Guide 33:2015 Reference materials – Good practice in using reference materials.
[8] 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 of Analysis CML-HRM-2005A/02 replaces Certificate CML-HRM2005A/01 issued on 11 May 2015.
Certificate of Analysis CML-HRM-2005A/03 replaces Certificate CML-HRM2005A/02 issued on 17 June 2016.
Certificate of Analysis CML-HRM-2005A/04 replaces Certificate CML-HRM2005A/03 issued on 11 April 2017.
Certificate of Analysis CML-HRM-2005A/05 replaces Certificate CML-HRM2005A/04 issued on 21 February 2018.
Certificate of Analysis CML-HRM-2005A/06 replaces Certificate CML-HRM2005A/05 issued on 02 May 2019.
Certificate of Analysis CML-HRM-2005A/07 replaces Certificate CML-HRM2005A/06 issued on 15 April 2020.
Certificate of Analysis CML-HRM-2005A/08 replaces Certificate CML-HRM2005A/07 issued on 11 May 2021.

Note

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

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