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Certificate of Analysis

CERTIFIED REFERENCE MATERIAL
HRM-2002A

Calcium, Potassium and Sodium in Frozen Human Serum

Batch Number
STY-0018-04
STY-0018-05
STY-0018-06

Foreword
A unit of the certified reference material (CRM) HRM-2002A consists of 1 ml each of three frozen human sera. Each serum contains three analytes (calcium, potassium and sodium) in different concentrations and is stored in glass vial with crimp-cap. The serum material appears as a transparent (or slightly cloudy) brownish yellow liquid after thawing.

HRM-2002A is 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-2002A 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 20.5°C (1.0220 g/ml, 1.0162 g/ml, and 1.0252 g/ml for STY-0018-04, STY-0018-05, and STY-0018-06, respectively) and the relative molecular masses of the analytes [40.08 (calcium), 39.10 (potassium) and 22.99 (sodium)]. The amount-of-substance concentration values are measured on a rational scale.

Table 1. Certified Concentration Values of Analytes ± Expanded Uncertainty (k = 2)

	STY-0018-04	STY-0018-05	STY-0018-06
Analyte	**(mmol/l)**	**(mmol/l)**	**(mmol/l)**
Calcium	2.56 ± 0.07	2.23 ± 0.07	2.71 ± 0.07
Potassium	4.54 ± 0.13	4.03 ± 0.10	4.89 ± 0.08
Sodium	139 ± 4	124 ± 4	153 ± 4

Each certified concentration value is the mean of measurements of at least five samples taken from a

minimum of four different 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 was determined using the isotope dilution mass spectrometry (IDMS) method.

The associated measurement uncertainty of each certified concentration value was estimated 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%.

Validity

The certified concentration values of HRM-2002A are valid within the specified measurement uncertainty until **12 Oct 2022**. The validity of HRM-2002A will be extended and all users will be informed if the certified values of the CRM are found to have changed or an updated COA may be issued. The certified concentration values of HRM-2002A 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. Extraneous calcium, potassium and sodium were added to achieve the desired high concentration pools.

Commutability

The methods used for the certification of HRM-2002A were all validated using the Standard Reference Material (SRM 956c) from NIST that was listed in the Joint Committee for Traceability in Laboratory Medicine (JCTLM) database.

In addition, all three frozen human sera of HRM-2002A were analysed by at least 22 clinical laboratories in Singapore in an External Quality Assessment (EQA) programme organised by HSA in 2012. 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-2002A.

Homogeneity

Homogeneity testing on HRM-2002A was performed on one sub-sample taken from ten bottles and five sub-samples taken from one bottle by external calibration method using inductively coupled plasma mass spectrometry (ICP-MS) for all three analytes. The sample size taken for homogeneity testing was 0.2 g. No significant differences in the between and within-bottle variances were found using *F*-test at 95 % confidence level. The Student's *t*-test also indicated no significant differences between the means obtained from between and within-bottle analyses. Thus, the serum materials were regarded as sufficiently homogeneous.

Stability

Stability testing for HRM-2002A was performed at – 80 ^{o}C on at least three occasions over a period of

at least 4 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.

Analytical Methods

For the determination of calcium and sodium, a multiple-point calibration standard addition method was used [6, 7]. 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, an exact matching isotope dilution mass spectrometry method was used [6]. The method involved, spiking with isotope labeled potassium, digestion with 5% HNO_3, followed by ICP-high resolution MS measurement.

Metrological Traceability

The certified concentration values are traceable to the International System of Units (SI) through the use of calcium, potassium and sodium SRMs from NIST (SRM 3109a for calcium, SRM 3141a for potassium, SRM 3152a and SRM 919b for sodium).

Intended Use

HRM-2002A is intended for use in the validation of methods or as quality control materials for the determination of calcium, potassium and sodium in human serum. Users may refer to ISO Guide 33:2015 [8] 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-2002A 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 [9].

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-2002A 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. 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.

Transport and Storage

HRM-2002A is transported in frozen state (in dry ice). Upon receipt, it should be stored at below – 60 °C. HRM-2002A 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.

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] Long, S. E.; Murphy, K. E.; NIST Special Publication 260-162, Government Printing Office: Washinton, DC (2006).
[7] Simpson, L. A.; Hearn. R.; Merson, S.; Catterick, T.; Talanta, 2005, 65, 900-906.
[8] ISO Guide 33:2015 Reference materials – Good practice in using reference materials.
[9] 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-J2002A/02 replaces Certificate CML-HRM-J2002A/01 issued on 12 April 2013.
Certificate of Analysis CML-HRM-J2002A/03 replaces Certificate CML-HRM-J2002A/02 issued on 04 October 2013.
Certificate of Analysis CML-HRM-J2002A/04 replaces Certificate CML-HRM-J2002A/03 issued on 17 March 2014.
Certificate of Analysis CML-HRM-J2002A/05 replaces Certificate CML-HRM-J2002A/04 issued on 12 June 2014.
Certificate of Analysis CML-HRM-J2002A/06 replaces Certificate CML-HRM-J2002A/05 issued on 07 Oct 2014.
Certificate of Analysis CML-HRM-J2002A/07 replaces Certificate CML-HRM-J2002A/06 issued on 23 Sep 2016.

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