

Certificate of Analysis

CERTIFIED REFERENCE MATERIAL HRM – 2011A

Sodium, Chloride, Copper, Selenium and Phosphorus in Human Serum

Batch Number

STY-0068-001

Description

A unit of the certified reference material (CRM) HRM-2011A consists of one vial (3 ml each) of human serum. Each serum is certified for five analytes (sodium, chloride, copper, selenium and phosphorus) and is stored in a glass vial with crimp-cap. The serum material appears as a transparent (or slightly cloudy) brownish yellow liquid after thawing.

HRM-2011A 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-2011A 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 23°C (1.0259 g/mL for STY-0068-001) and the relative molecular masses of the analytes [22.99 (sodium), 35.45 (chloride), 30.97 (phosphorus), 63.55 (copper), and 78.97 (selenium)].

Table 1. Certified Concentration Values of Analytes

Analyte	mg/kg	mmol/l
Sodium	3357 ± 80	149.8 ± 3.6
Chloride	3864 ± 76	111.8 ± 2.2
Phosphorus	125.7 ± 7.2	4.17 ± 0.24

Table 2. Certified Concentration Values of Analytes

Analyte	mg/kg	µmol/l
Copper	1.148 ± 0.039	18.53 ± 0.64
Selenium	0.1282 ± 0.0052	1.666 ± 0.068

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

Each certified concentration value is the mean of measurements of at least eight samples taken from a minimum of two different bottles. The certified mass fraction for sodium was determined by internal standard method using inductively coupled plasma-mass spectrometry (ICP-MS) [4]. The certified mass fraction for chloride, copper and selenium were determined by isotope dilution mass spectrometry (IDMS) method using ICP-MS or inductively coupled plasma high resolution mass spectrometry (ICP-HR-MS) [5]. The certified mass fraction for phosphorus was determined by standard addition method using ICP-HR-MS [6].

The associated measurement uncertainty of each certified concentration value was estimated in accordance with ISO/IEC Guide 98-3:2008 Uncertainty of Measurement – Part 3: Guide to the Expression of Uncertainty in Measurement (GUM:1995) [7]. The expanded uncertainty [coverage factor (k) of 2] corresponded to a level of confidence of about 95 %.

Homogeneity

Homogeneity testing on HRM-2011A was performed on two sub-samples taken from eleven bottles each by using ICP-MS. 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-2011A was performed at storage temperature below -60 °C on at least four occasions over a period of up to 12 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 estimated from the standard error of the slope.

Validity of Concentration Values

The certified concentration values of HRM-2011A are valid within the specified measurement uncertainties until **09 Jun 2026**. The validity of HRM-2011A will be extended if it is tested to be sufficiently stable for continuous use. The certified concentration values of HRM-2011A are invalid when the serum material has deteriorated or is mishandled.

Commutability

The methods used for the certification of HRM-2011A were all validated using the Standard Reference Materials (SRM 956c and ERM-DA120a) that are listed in the Joint Committee for Traceability in Laboratory Medicine (JCTLM) database.

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-2011A.

Analytical Methods

For the determination of sodium, internal standard method was used. The method involved spiking of aluminium internal standard and digestion with 5% HNO₃, followed by ICP-MS measurement.

For the determination of chloride, an exact matching IDMS method was used. The method involved spiking with isotope labeled standard (³⁷Cl from Oak Ridge National Laboratory). The proteins were removed using ammonium molybdate and chloride was precipitated using silver nitrate. The silver chloride was redissolved in concentrated ammonia, diluted with Type I water, followed by ICP-HR-MS measurement.

For the determination of copper, an exact-matching IDMS method was used. The method involved spiking with isotope labeled standard (⁶⁵Cu from Oak Ridge National Laboratory) and digestion with

concentrated HNO₃. The blend was then diluted with Type I water, followed by ICP-MS measurement.

For the determination of selenium, an exact-matching IDMS method was used. The method involved spiking with isotope labeled standard (⁷⁷Se from Oak Ridge National Laboratory) and digestion with concentrated HNO₃/H₂O₂ at 85 °C. The blend was then diluted with Type I water and methanol (3%), followed by ICP-MS measurement.

For the determination of phosphorus, standard addition method was used. The sample was microwave digested using HNO₃ and H₂O₂. The method involved spiking of germanium internal standard. Different amounts of phosphorus standard were then spiked into the sample to produce sample blends, 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 919b for sodium and chloride, SRM 3114 for copper, SRM 3149 for selenium and SRM 3139a for phosphorus).

Intended Use

HRM-2011A is intended for use in the validation of methods or as quality control materials for the determination of sodium, chloride, copper, selenium and phosphorus in human serum. Users may refer to ISO Guide 33:2000 [8] for the recommended statistical treatment of the certified reference values and the associated uncertainties of the CRM as control materials.

Instruction for Use

The human serum materials were prepared by a commercial supplier of human blood products. 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-2011A should be thawed at room temperature (between 18 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. It is not advisable to re-thaw the materials for more than two times and to keep opened bottles for a prolonged period of time.

The minimum sample size should be 0.1 g. The certified concentration values may not be valid if smaller amounts are taken.

Storage

HRM-2011A is transported in frozen state (in dry ice). Upon receipt, it should be stored at below -60 °C. The material has been shown to be stable at -20 °C for up to eight weeks. HRM-2011A 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 mass fractions of the analytes.

Safety Precautions for Users

HRM-2011A 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].

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] Kramer, U.; Kress, M.; Reinauer, H.; Spannagl, M.; Kaiser, P.; Candidate Reference Measurement Procedures for Chloride, Potassium, Sodium, Calcium, Magnesium, and Lithium by Inductively Coupled Plasma (Isotope Dilution) Sector Field Mass Spectrometry; Clin. Lab. (2013) 59: 1017-1029.
- [5] Sargent, M.; Harrington, C.; Harte, R.; Guidelines for Achieving High Accuracy in Isotope Dilution Mass Spectrometry, RSC Publishing, 2002.
- [6] Ng, S. Y.; Dewi, F.; Wang, J.; Sim, L. P.; Shin, R. Y. C.; Lee, T. K.; Development of a Cosmetic Cream Certified Reference Material: Certification of Lead, Mercury and Arsenic Mass Fractions in Cosmetic Cream; Int. J. Mass Spectrom. (2015) 389: 59-65.
- [7] ISO/IEC Guide 98-3:2008 Uncertainty of measurement – Part 3: Guide to the expression of uncertainty in measurement (GUM: 1995).
- [8] ISO Guide 33:2000 Uses of certified 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-2011A/02 replaces Certificate CML-HRM-2011A/01 issued on 9 June 2017.

Certificate of Analysis CML-HRM-2011A/03 replaces Certificate CML-HRM-2011A/02 issued on 12 October 2017.

Certificate of Analysis CML-HRM-2011A/04 replaces Certificate CML-HRM-2011A/03 issued on 21 February 2018.

Certificate of Analysis CML-HRM-2011A/05 replaces Certificate CML-HRM-2011A/04 issued on 8 May 2018.

Certificate of Analysis CML-HRM-2011A/06 replaces Certificate CML-HRM-2011A/05 issued on 7 June 2019.

Certificate of Analysis CML-HRM-2011A/07 replaces Certificate CML-HRM-2011A/06 issued on 7 July 2020.

Certificate of Analysis CML-HRM-2011A/08 replaces Certificate CML-HRM-2011A/07 issued on 7 June 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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