

Certificate of Analysis

CERTIFIED REFERENCE MATERIAL HRM – 2023A

Inorganic Phosphate in Human Serum

Batch Number

STY-0068-001

Description

A unit of the certified reference material (CRM) HRM-2023A consists of one vial (3 mL each) of human serum. Each serum is certified for inorganic phosphate 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.

The CRM is produced with reference to the requirements set out in ISO/IEC 17025:2017 [1], ISO 17034:2016 [2] and ISO 33405:2024 [3].

Certified Concentration Value

The certified concentration value for inorganic phosphate in HRM-2023A is provided in Table 1. This value was calculated from mass fraction value, the measured serum density at 23°C (1.0259 g/mL for STY-0068-001) and the relative molecular mass of phosphate, 94.97.

Table 1. Certified Concentration Value	
Analyte	mmol/L
Inorganic phosphate	1.120 ± 0.023

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

The certified concentration value represents the mean of measurements obtained from six samples taken from two different vials. The certified concentration for inorganic phosphate was determined by gravimetric external calibration method using ion chromatography with suppressed conductivity detection (IC-CD).

The associated measurement uncertainty of the certified concentration value was estimated in accordance with 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 of inorganic phosphate was performed on two sub-samples taken from eight bottles (a total of 16 sub-samples) by using Roche Cobas C311 clinical analyser. The sample size taken for homogeneity testing was 2.5 µL. No significant differences in the between and within-bottle variances were found using one-way ANOVA at 95 % confidence level. Thus, the material was regarded to be sufficiently homogeneous. The u_{bb} was evaluated from the uncertainty due to between-bottle inhomogeneity [3].

Stability

Stability testing of inorganic phosphate was performed at storage temperature below -60 °C on at least three occasions over a period of up to 20 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 value is valid within the specified measurement uncertainties until **30 September 2027**. The validity of HRM-2023A will be extended if it is tested to be sufficiently stable for continuous use. The certified concentration value of HRM-2023A is invalid when the serum material has deteriorated or is mishandled.

Commutability

Commutability study of inorganic phosphate in HRM-2023A was conducted by analysing 28 patient serum samples and the CRM by both gravimetric external calibration method using IC-CD and routine colorimetric methods using Roche Cobas c311 and Abbott Alinity C clinical analysers. The commutability of HRM-2023A on different analysers were evaluated using the linear regression model suggested by CLSI [5,6]. The results demonstrated satisfactory commutability of inorganic phosphate in HRM-2023A for these clinical analysers.

Analytical Method

The method used for the certification of HRM-2023A was validated using NIST SRM 956d Electrolytes in Frozen Human Serum (three concentration levels), sourced from the National Institute of Standards and Technology (NIST) and spiked recovery experiments using NIST SRM 956d materials. Samples were diluted with Milli-Q water and inorganic phosphate mass fractions were determined from the calibration curve. The calibration curve was constructed by plotting mean peak areas of five gravimetrically prepared calibration standards against inorganic phosphate mass fraction (mg/kg), and this regression was used for quantification of inorganic phosphate in HRM-2023A. A linear regression with a goodness-of-fit of (R^2) ≥ 0.999 was required as the acceptance criterion before proceeding with sample quantification.

Metrological Traceability

The certified concentration value is traceable to the International System of units (SI) through the use of phosphate anion standard solution (SRM 3186) from NIST.

Intended Use

HRM-2023A is a secondary reference material intended for use in the validation of methods or as quality control materials for the determination of inorganic phosphate in human serum. Users may refer to ISO 33403:2024 [7] 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-2023A 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.

The recommended minimum sample size is 2.5 µL. The certified values may not be valid for sample portions smaller than the recommended minimum. In addition, the certified value do not apply to previously re-thawed and opened vials, as the stability of CRM under such conditions has not been investigated.

Transport and Storage

HRM-2023A is transported in frozen state (in dry ice). Upon receipt, it should be stored at below -60 °C. Exposure to sunlight or ultraviolet radiation should be avoided. Storage of the thawed material at room temperature or in the refrigerator may result in changes in the mass fraction of the analyte.

Safety Precautions for Users

HRM-2023A 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]. The Safety Data Scheet (SDS) for this material is provided separately and contains essential safety and handling information.

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 33405:2024 Reference materials – Approaches 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] CLSI. Characterisation and qualification of commutable reference materials for laboratory medicine; Approved Guideline. CLSI document EP30-A. Wayne: Clinical and Laboratory Standards Institute; 2010.
- [6] CLSI. Evaluation of Commutability of Processed Samples. 4th ed. CLSI guideline EP14. Clinical and Laboratory Standards Institute; 2022.
- [7] ISO 33403:2024 Reference materials – Requirements and recommendations for use.
- [8] Meechan, Paul J., and Jeffrey Potts Biosafety in Microbiological and Biomedical Laboratories: 6th Edition 2020. Centers for Disease Control and Prevention (U.S.), 2020, Stacks. <https://stacks.cdc.gov/view/cdc/97733>.

Certificate Revision Records

Certificate Ref. No.	Date of issue	Reason for issuance
CML-HRM-2023A/01	30 September 2026	Issuance of first certificate

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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