National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 956d

Electrolytes in Frozen Human Serum

This Standard Reference Material (SRM) is primarily intended for use in the calibration and validation of procedures and methods employed in clinical analysis for the determination of electrolytes in either diluted or undiluted human serum or plasma. This SRM can be used for calibrating direct-reading ion-selective electrode (ISE) analyzers [1] and for quality assurance in validating secondary reference materials. A unit of SRM 956d consists of six sealed borosilicate glass ampoules of frozen human serum, two ampoules each of three different concentration levels. Each ampoule contains approximately 2 mL of human serum.

Certified Values: The certified concentration values of the electrolytes in each level of the material are listed in Table 1, and represent the means of results based on measurements using a single primary method, with the exception of sodium. The certified concentrations for calcium (Ca), lithium (Li), magnesium (Mg), and potassium (K) are based on measurements using isotope dilution-inductively coupled plasma-mass spectrometry (ID-ICP-MS) [2–5]. The certified concentrations for chloride are based on measurements using micro-coulometry [4]. The certified concentrations for sodium are based on inductively coupled plasma-optical emission spectrometry (ICP-OES) and ISE potentiometry. All analyte values are certified as total element. For convenience, the certified concentration values are expressed in both units of mmol/L and mg/dL. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [6]. The measurands are the concentrations of the electrolytes reported in Table 1. Metrological traceability is to the SI derived unit for mass concentration (expressed as milligrams per deciliter) and amount-of-substance concentration (expressed as millimoles per liter).

Reference Values: Reference concentration values for ionized calcium (Ca^{2+}), phosphate (PO_4^{3-}) and total phosphorus (P) are provided in Table 2. The reference values for ionized calcium are based on ISE potentiometry following the protocol described in the approved Clinical and Laboratory Standards Institute (CLSI) Designated Comparison Method (DCM) [7]. Reference values are noncertified values that are the best estimate of the true value; however, the values do not meet NIST criteria for certification and are provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods [6]. The measurands are the concentrations of the electrolytes reported in Table 2 using the methods indicated in the text. Metrological traceability is to the SI derived unit for mass concentration (expressed as milligrams per deciliter) and amount-of-substance concentration (expressed as millimoles per liter).

Expiration of Certification: The certification of **SRM 956d** is valid, within the measurement uncertainty specified, until **01 April 2023**, provided the SRM is handled and stored in accordance with instructions given in this certificate (see "Instructions for Storage and Use"). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Acquisition of the material and coordination of measurements leading to the certification of this SRM were performed by S.E. Long, and L.L. Yu of the NIST Chemical Sciences Division.

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Robert L. Watters, Jr., Director
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Analytical measurements at NIST were performed by B.L. Catron, W. C. Davis, R. Easley, S.E. Long, K.W. Pratt, S.A. Rabb, T.W. Vetter and L.L. Yu of the NIST Chemical Sciences Division.

Additional measurements of ionized calcium were made at Abbott Point of Care (Ontario, Canada) by M. Glenn, D. Padavan, and S. Breeze. Additional measurements of phosphate were made at Roche Diagnostics (Indianapolis, IN) and were coordinated by C. Cañada Vilalta and M. Swartzentruber.

Statistical analysis of the data was performed by C. Hagwood of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

NOTICE AND WARNINGS TO USERS

SRM 956d IS INTENDED FOR RESEARCH USE. THIS IS A HUMAN SOURCE MATERIAL. HANDLE PRODUCT AS A BIOHAZARDOUS MATERIAL CAPABLE OF TRANSMITTING INFECTIOUS DISEASE. The supplier of this serum has reported that each donor unit of serum or plasma used in the preparation of this product has been tested by FDA-approved methods and found to be non-reactive/negative for human immunodeficiency virus (HIV) 1 and 2 antibodies, hepatitis B surface antigen (HbsAg), hepatitis C virus (HCV), and syphilis. However, no known test method can offer complete assurance that hepatitis B virus, HCV, HIV, or other infectious agents are absent from this material. Accordingly, this human blood-based product should be handled at the Biosafety Level 2 or higher as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health Manual [8].

INSTRUCTIONS FOR STORAGE AND USE

Stability and Storage: The serum is shipped frozen (on dry ice) and, upon receipt, should be immediately stored frozen until needed for use. A freezer temperature of –20 °C is acceptable for storage up to one week. If a longer storage time is anticipated, the material should be stored at or below –50 °C. The SRM should not be exposed to sunlight or ultraviolet radiation. Storage of thawed material at room or refrigerator temperature may result in changes to the analyte concentrations.

Use: *For analysis of all electrolytes except ionized calcium:* Each ampoule should be inspected carefully for circular cracks at the base. If the ampoule is cracked, or has visible deposits of serum material on the outside, it should not be used. The serum in intact ampoules should be thawed to room temperature, and mixed by inverting gently at least five times before sampling. When opening ampoules, wear appropriate eye protection, gloves, and protective clothing. Check that all of the liquid has drained out of the neck of the ampoule. If needed, gently tap the neck to facilitate drainage. **NOTE THAT AMPOULES ARE NOT PRESCORED**. To open, wear protective gloves to avoid injury, score around the narrowest part of the neck with a file or other suitable device, and snap open. Ampoules should not be resealed. Once opened, the contents of the ampoule should be used as soon as possible.

Use: *For analysis of ionized calcium:* Because of the influence of pH on ionized calcium, it is important that the samples be thawed and re-equilibrated with the gas in the ampoule headspace using the specified conditions given below [7].

1. Remove samples from freezer and thaw at ambient temperature for 1 hour and 40 minutes. **NOTE:** Ambient temperature must be between 20 °C to 24 °C.
2. During the first few minutes of thawing, inspect ampoules carefully for cracks or breaks. Ampoules that are cracked or broken should be appropriately discarded.
3. After the 1 hour and 40 minutes thawing period, shake each ampoule vigorously with an up and down motion along the cylindrical axis for 10 seconds to create foam.
4. Wait an additional 30 minutes after shaking, then begin analyzing the samples.
5. Open the ampoule and aspirate the sample from as close as possible to the bottom of the ampoule. The sample must be introduced into the analyzer within one minute of opening the ampoule.
6. If it is not possible to aspirate sample directly from the ampoule into the analyzer for the particular system being used, the sample may be aspirated into a syringe while minimizing contact with air. **NOTE:** The sample should be analyzed within one minute of opening the ampoule.

Density: The mean densities and expanded uncertainties (k = 2) at 22.00 ºC were determined by digital density meter and are provided to allow conversions between results expressed in mass fraction and concentration. The density values are 1.0229 g/mL ± 0.0006 g/mL for Level 1, 1.0234 g/mL ± 0.0006 g/mL for Level 2, and 1.0241 g/mL ± 0.0006 g/mL for Level 3.

Homogeneity Assessment: The homogeneity of all analytes was assessed by the supplier of the material. Analysis of variance did not show statistically significant heterogeneity.

SOURCE AND PREPARATION OF SERUM POOLS

SRM 956d was prepared by Aalto Scientific, Ltd (Carlsbad, CA).(1) The material was prepared from pooled units of normal human serum, and its appearance is a clear amber liquid, free of particulate matter. Donor units were collected at an FDA licensed blood collection facility and allowed to clot for a minimum of two hours at room temperature using no additives to assist in the clotting process. The serum pool was frozen at –20 °C, thawed, and filtered through an Avicel cellulose slurry under vacuum to remove fibrin. Gentamicin sulfate was added as an antibacterial agent. The filtered base pool was diluted with a sodium bicarbonate solution to adjust the potassium level. The serum was then filtered through a pre-sterilized 0.22 µm filter. The appropriate amounts of American Chemical Society (ACS) grade chloride salts were added to the Level 1 and Level 3 subpools to adjust the concentrations of sodium, potassium, calcium, magnesium, phosphorus and lithium to the desired levels. The Level 2 subpool was prepared by gravimetrically combining equal amounts of the Level 1 and Level 3 subpools, then adjusting with the appropriate salts as needed. The pH was adjusted to 7.4 at 37 °C. Finally, 2.0 mL aliquots of each subpool were dispensed into Wheaton un-scored glass ampoules, flushed with an inert gas plus 5 % CO_2 overlay, flame sealed, and stored at –70 °C.

Table 1. Certified Values[a] for Electrolytes in SRM 956d

Electrolyte	Molar Concentration (mmol/L)	Mass Concentration (mg/dL)	Coverage Factor, *k*
Level 1			
Calcium	3.407 ± 0.030	13.65 ± 0.12	2.00
Chloride	94.53 ± 0.21	335.1 ± 0.8	2.00
Lithium	1.965 ± 0.029	1.364 ± 0.020	2.00
Magnesium	1.471 ± 0.010	3.576 ± 0.025	2.00
Potassium	5.752 ± 0.049	22.49 ± 0.19	2.00
Sodium	120.0 ± 0.7	275.9 ± 1.6	2.00
Level 2			
Calcium	2.857 ± 0.025	11.45 ± 0.10	2.00
Chloride	108.5 ± 0.2	384.7 ± 0.8	2.00
Lithium	1.207 ± 0.020	0.838 ± 0.014	2.00
Magnesium	0.961 ± 0.007	2.336 ± 0.016	2.00
Potassium	3.730 ± 0.035	14.58 ± 0.14	2.00
Sodium	139.3 ± 0.9	320.2 ± 2.0	2.00
Level 3			
Calcium	2.282 ± 0.018	9.145 ± 0.072	2.00
Chloride	122.6 ± 0.3	434.8 ± 0.9	2.00
Lithium	0.455 ± 0.012	0.316 ± 0.008	2.00
Magnesium	0.434 ± 0.003	1.055 ± 0.008	2.00
Potassium	1.611 ± 0.014	6.298 ± 0.054	2.00
Sodium	158.7 ± 1.1	364.8 ± 2.4	2.00

[a] Each certified value is the mean or combination of means of results provided by ID-ICP-MS, ICP-OES, micro-coulometry, and ISE potentiometry. The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence, consistent with the ISO/JCGM Guide [9]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is the combined uncertainty that incorporates within-method uncertainty and Type B uncertainty components related to the analysis, and k is the coverage factor corresponding to approximately 95 % confidence for each analyte [9]. For estimates based on multiple methods, the combined uncertainty also incorporates between-methods uncertainty.

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(1) Certain commercial equipment, instruments, or materials are identified in this certificate in order to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Table 2. Reference Values(a) for Electrolytes in SRM 956d

Electrolyte	Molar Concentration (mmol/L)	Mass Concentration (mg/dL)	Coverage Factor, k
Level 1			
Ionized Calcium	1.77 ± 0.07	7.08 ± 0.29	2.00
Phosphate	1.99 ± 0.03	6.17 ± 0.09	2.00
Phosphorus	4.247 ± 0.029	13.15 ± 0.09	2.21
Level 2			
Ionized Calcium	1.47 ± 0.03	5.91 ± 0.11	2.00
Phosphate	1.52 ± 0.02	4.71 ± 0.07	2.00
Phosphorus	3.782 ± 0.023	11.71 ± 0.07	2.18
Level 3			
Ionized Calcium	1.17 ± 0.03	4.68 ± 0.11	2.00
Phosphate	1.05 ± 0.01	3.24 ± 0.03	2.00
Phosphorus	3.279 ± 0.017	10.16 ± 0.05	2.13

(a) The reference values are the means of results from a single method. The uncertainty provided is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence, consistent with the ISO/JCGM Guide [9]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is the combined uncertainty that incorporates within-method uncertainty and Type B uncertainty components related to the analysis, and k is the coverage factor corresponding to approximately 95 % confidence for each analyte [9].

REFERENCES

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Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-2200; fax (301) 948-3730; e-mail srminfo@nist.gov.