National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 956c

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 956c consists of six sealed ampoules of frozen human serum, two ampoules each of three different concentration levels. Each ampoule contains approximately 2.0 mL of human serum.

Certified Concentration Values: The certified concentrations 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 and chloride. The certified concentrations for calcium, lithium, magnesium and potassium 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] and ISE potentiometry. The certified concentrations for sodium are based on high-performance inductively coupled plasma – optical emission spectrometry (ICP-OES) and ISE potentiometry. All analyte concentrations 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]. Uncertainties and effective degrees of freedom, necessary for propagation of uncertainties if this SRM is used for calibration, are provided in Table 2.

Reference Concentration Values: Reference concentration values for ionized calcium are provided in Table 3. The method of analysis for ionized calcium is ISE potentiometry following the protocol described in the approved NCCLS Designated Comparison Method (DCM) [7]. Ionized calcium measurements were made by the Mayo Clinic (Rochester, MN) using two different calcium-selective membranes. 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].

Expiration of Certification: The certification of **SRM 956c** is valid, within the measurement uncertainty specified, until **01 September 2018**, provided the SRM is handled in accordance with instructions given in this certificate (see "Instructions for 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) will facilitate notification.

The technical project leader responsible for the coordination of measurements leading to the certification of this SRM was S.E. Long of the NIST Analytical Chemistry Division.

Analytical measurements at NIST were performed by T.A. Butler, S.E. Long, K.E. Murphy, K.W. Pratt, S.A. Rabb, and T.W. Vetter of the NIST Analytical Chemistry Division. Additional measurements of chloride and ionized calcium at the Mayo Clinic were coordinated by B. Karon, T. Milz, and A. Saenger.

Statistical consultation and data evaluation for all electrolytes were provided by C. Hagwood of the NIST Statistical Engineering Division.

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Support aspects involved in the issuance of this SRM were coordinated through the NIST Measurement Services Division.

NOTICE AND WARNING TO USERS

SRM 956c IS INTENDED FOR IN-VITRO DIAGNOSTIC USE ONLY. 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 an FDA-approved method and found 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, hepatitis C virus, 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].

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 in the analyte concentrations.

INSTRUCTIONS FOR USE FOR ANALYSIS OF ALL ELECTROLYTES EXCEPT IONIZED CALCIUM

Place the ampoule to be used inside another container, such as a plastic beaker, to ensure containment of the serum in case the ampoule cracks. 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. Open the pre-scored ampoule by snapping off the top at the narrowest segment of the neck. Ampoules should not be resealed. Once opened, the contents of the ampoule should be used as soon as possible.

Transfer the solution from the ampoule using a suitable transfer pipette. DO NOT PIPETTE BY MOUTH. Pouring solution out of the ampoule is not recommended as the narrow cross section of the neck does not facilitate easy exchange of liquid and air.

The mean densities and expanded uncertainties ($k = 2$) at 22.4 ºC were determined by digital density meter to be 1.0229 g/mL ± 0.0001 g/mL for Level 1, 1.0241 g/mL ± 0.0001 g/mL for Level 2 and 1.0252 g/mL ± 0.0001 g/mL for Level 3, and are provided to allow conversions between results expressed in per mass and per volume.

SPECIAL SAMPLE HANDLING INSTRUCTIONS FOR MEASUREMENT OF IONIZED CALCIUM

Because of the influence of pH on ionized calcium, it is important that the samples are 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 of 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.

SOURCE AND PREPARATION OF SERUM POOLS[1]

SRM 956c was prepared by Aalto Scientific, Ltd, Carlsbad, CA. 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 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 plasma 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 I and Level III subpools to adjust the concentrations of sodium, potassium, calcium, magnesium, and lithium to the desired levels. The Level II subpool was made from equal amounts of the Level I and Level III subpools. The pH was adjusted to 7.4 at 37 °C. All levels were further filtered through a sterilized 0.2 µm filter. Finally, 2.0 mL aliquots of each subpool were dispensed into Wheaton pre-scored glass ampoules flushed with an inert gas plus 5 % CO_2 overlay, flame sealed, and stored at –70 °C.

[1]Certain commercial equipment, instruments, or materials are identified in this certificate in order to specify adequately 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 1. Certified Concentrations and Uncertainties[(a)] for Electrolytes (in mmol/L and mg/dL)

Electrolyte	Level I		Level 2		Level 3	
	mmol/L	mg/dL	mmol/L	mg/dL	mmol/L	mg/dL
Total Calcium	2.981 ± 0.022	11.95 ± 0.09	2.538 ± 0.016	10.17 ± 0.06	2.095 ± 0.013	8.395 ± 0.053
Chloride	104.9 ± 3.2	371.8 ± 11.3	121.5 ± 2.5	430.7 ± 8.7	137.4 ± 1.8	487.2 ± 6.5
Lithium	1.606 ± 0.024	1.114 ± 0.017	1.068 ± 0.016	0.741 ± 0.011	0.457 ± 0.007	0.317 ± 0.005
Magnesium	1.247 ± 0.013	3.031 ± 0.032	0.857 ± 0.010	2.084 ± 0.023	0.470 ± 0.005	1.143 ± 0.013
Potassium	5.976 ± 0.051	23.36 ± 0.20	3.977 ± 0.034	15.55 ± 0.13	1.982 ± 0.017	7.748 ± 0.066
Sodium	118.8 ± 1.0	273.2 ± 2.3	137.5 ± 1.6	316.1 ± 3.7	157.4 ± 1.4	361.7 ± 3.3

[(a)] The uncertainty in the certified value is calculated as $U = ku_c$, where u_c is the combined standard uncertainty calculated according to the ISO Guide [9] and k is the coverage factor. The value of u_c is intended to represent, at the level of one standard deviation, the combined effect of uncertainty components associated with the measurement uncertainty and additional Type B uncertainties. The expanded uncertainty, $U = ku_c$, is defined as an interval estimated to have a level of confidence of 95 %. For users to propagate the uncertainty of calibration when SRM 956c is used as a calibrant, the combined standard uncertainty, u_c, and its associated effective degrees of freedom, ν_{eff}, for each level of each analyte concentration are listed in Table 2.

Table 2. Combined Standard Uncertainties (mmol/L) and Effective Degrees of Freedom for Electrolytes

Electrolyte	Level 1		Level 2		Level 3	
	u_c	ν_{eff}	u_c	ν_{eff}	u_c	ν_{eff}
Total Calcium	0.011	100	0.0081	51	0.0066	50
Chloride	1.60	56	1.22	49	0.86	16
Lithium	0.012	243	0.0082	235	0.0035	241
Magnesium	0.0055	6.2	0.0042	9.2	0.0024	10.1
Potassium	0.022	7.7	0.014	7.1	0.0072	7.2
Sodium	0.39	5.4	0.67	7.1	0.57	5.5

Table 3. Reference Concentrations (mmol/L and mg/dL)
for Ionized Calcium in SRM 956c

		Level 1	Level 2	Level 3
Ionized Calcium [(a)]	mmol/L	1.78 ± 0.08	1.48 ± 0.07	1.19 ± 0.05
	mg/dL	7.12 ± 0.31	5.95 ± 0.26	4.76 ± 0.21

[(a)] Reference values are based on results from a participating external laboratory and the uncertainties represent the expanded uncertainties calculated as $U = ku_c$, where u_c is the combined standard uncertainty calculated according to the ISO Guide [9] and k is the coverage factor. The expanded uncertainty is expressed as a 95 % level of confidence, with $k = 2.0$.

REFERENCES

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[9] JCGM 100:2008; *Guide to the Expression of Uncertainty in Measurement*; (ISO GUM 1995 with Minor Corrections), Joint Committee for Guides in Metrology: BIPM, Sèvres Cedex, France (2008); available at http://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf; see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297; U.S. Government Printing Office: Washington, DC (1994); available at http://physics.nist.gov/Pubs/.

Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-2200; fax (301) 926-4751; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.