National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 956b

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 analyzers [1] and for validating secondary reference materials. A unit of SRM 956b 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 serum analytes and density for each concentration level of the material are listed in Table 1, and represent the means of results based on measurements using a single primary method. 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 accounted for by NIST. All analyte concentrations are certified as totals. The certified concentrations for calcium, lithium, magnesium and potassium are based on measurements using isotope dilution - inductively coupled plasma - mass spectrometry (ID-ICP-MS) [2–4]. The certified concentrations for chloride are based on measurements using isotope dilution – thermal ionization mass spectrometry (ID-TIMS) [4,5]. The certified concentrations for sodium are based on inductively coupled plasma – mass spectrometry (ICP-MS) with stable isotope internal standard [6]. Density is determined using a semi-micro gravimetric method [7]. For convenience, the certified concentration data are expressed both in units of mmol/L and mg/dL.

Reference Concentration Values: Reference concentration values for ionized calcium are provided in Table 3. The method of analysis for ionized calcium is ion selective electrode (ISE) potentiometry following the protocol described in the approved NCCLS Designated Comparison Method (DCM) [8]. Ionized calcium measurements were made by the Mayo Clinic (Rochester, MN) using three 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.

Expiration of Certification: The certification of this SRM is valid within the measurement uncertainties specified, until **01 September 2014,** provided the SRM is handled and stored in accordance with the instructions given in this certificate (see "Instructions for Use"). The certification is nullified if the SRM is contaminated or 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 all electrolytes was S.E. Long of the NIST Analytical Chemistry Division.

Stephen A. Wise, Chief
Analytical Chemistry Division

Gaithersburg, MD 20899
Certificate Issue Date: 12 September 2006
See Certificate Revision History on Last Page

Robert L. Watters, Jr., Chief
Measurement Services Division

Analytical measurements at NIST were performed by S.E. Long, J.L. Mann, K.E. Murphy and R.D. Vocke, Jr. of the NIST Analytical Chemistry Division. Additional measurements at the Mayo Clinic were performed by John Butz and Mary Burritt.

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

The support aspects involved in the issuance of this SRM were coordinated through the Measurement Services Division.

NOTICE AND WARNING TO USERS

SRM 956b 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 HIV-1 & 2 antibodies, HbsAg, 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 [9].

Stability and Storage: The serum is shipped frozen (on dry ice) and, upon receipt, should be stored frozen until ready for use. A freezer temperature of −20 ºC is acceptable for storage up to one week. If a longer storage time is anticipated, the material should be stored at or below −50 ºC. 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.

Table 1a. Certified Concentrations and Uncertainties[(a)] for Electrolytes: Level I (in mmol/L and mg/dL)

Electrolyte	mmol/L	mg/dL
Total Calcium	2.949 ± 0.019	11.82 ± 0.08
Chloride	99.01 ± 0.66	351.0 ± 2.3
Lithium	1.920 ± 0.027	1.333 ± 0.019
Magnesium	1.522 ± 0.020	3.699 ± 0.048
Potassium	5.973 ± 0.045	23.35 ± 0.18
Sodium	120.1 ± 1.4	276.1 ± 3.1
Density (g/mL) at 22 ºC		1.024 ± 0.001

Table 1b. Certified Concentrations and Uncertainties[(a)] for Electrolytes: Level 2 (in mmol/L and mg/dL)

Electrolyte	mmol/L	mg/dL
Total Calcium	2.456 ± 0.015	9.844 ± 0.060
Chloride	111.88 ± 0.82	396.7 ± 2.9
Lithium	1.207 ± 0.017	0.838 ± 0.012
Magnesium	0.994 ± 0.013	2.417 ± 0.031
Potassium	3.983 ± 0.029	15.57 ± 0.11
Sodium	141.0 ± 1.6	324.2 ± 3.6
Density (g/mL) at 22 ºC		1.025 ± 0.001

Table 1c. Certified Concentrations and Uncertainties[(a)] for Electrolytes: Level 3 (in mmol/L and mg/dL)

Electrolyte	mmol/L	mg/dL
Total Calcium	1.974 ± 0.013	7.911 ± 0.052
Chloride	126.85 ± 0.81	449.7 ± 2.9
Lithium	0.486 ± 0.007	0.337 ± 0.005
Magnesium	0.458 ± 0.006	1.113 ± 0.014
Potassium	1.987 ± 0.014	7.768 ± 0.055
Sodium	160.7 ± 1.8	369.4 ± 4.2
Density (g/mL) at 22 ºC		1.025 ± 0.001

[(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 and NIST Guides [10] 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 956b is used as a calibrant, the combined standard uncertainty, u_c, and its associated effective degrees of freedom, v_{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	v_{eff}	u_c	v_{eff}	u_c	v_{eff}
Total Calcium	0.0096	51	0.0073	158	0.0066	58
Chloride	0.29	8	0.35	7	0.35	8
Lithium	0.014	13570	0.0086	261200	0.0035	333250
Magnesium	0.010	3678	0.0065	2713	0.0030	28512
Potassium	0.022	191	0.014	4118	0.0072	2534
Sodium	0.69	716	0.80	840	0.93	561

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

		Level 1	Level 2	Level 3
Ionized Calcium [(a)]	mmol/L	1.71 ± 0.08	1.37 ± 0.07	1.09 ± 0.05
	mg/dL	6.87 ± 0.33	5.48 ± 0.28	4.38 ± 0.21

[(a)] The results are expressed as the reference value ± the expanded uncertainty. The expanded uncertainty is expressed as a 95 % confidence interval.

INSTRUCTIONS FOR USE FOR 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 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.

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 [8].

1. Remove samples from freezer and thaw at ambient temperature for 1 hour and 40 minutes. **NOTE:** Ambient temperature must be between 20 ºC to 24 ºC.
2. During the first few minutes of thawing, inspect ampoules carefully for cracks or breaks. Ampoules that are cracked or broken should be 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 956b was prepared by EURO-TROL b.v. (Wageningen, The Netherlands). 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 clot process. The serum pool was frozen at –20 ºC, 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 ºC. Finally, 2.0 mL aliquots of each subpool were dispensed into 4.5 mL Duran glass ampoules flushed with an inert gas plus 5 % CO_2 overlay, flame sealed, and stored at –50 ºC.

REFERENCES

[1] NCCLS; *Standardization of Sodium and Potassium Ion-Selective Electrode Systems to the Flame Photometric Reference Method*; Approved Standard, NCCLS Document C29-A2 (ISBN 1-56238-410-4), Wayne, PA (2000).

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[8] NCCLS, *A Designated Comparison Method for the Measurement of Ionized Calcium in Serum*; Approved Standard, NCCLS Document C39-A (ISBN 1-56238-367-1) Wayne, PA (2000).

[9] U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories*; U.S. Government Printing Office: Washington, DC (1988).

[10] ISO; *Guide to the Expression of Uncertainty in Measurement*; ISBN 92-67-10188-9, 1st ed., International Organization for Standardization: Geneva, Switzerland (1993); see 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).

Certificate Revision History: 12 September 2006 (This revision reports the addition of the certified value for chloride); 12 October 2004 (Original certificate date).

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) 975-6776; fax (301) 926-4751; e-mail srminfo@nist.gov; or via at the Internet at http://www.nist.gov/srm.

[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.