



Certificate of Analysis

Standard Reference Material[®] 956a

Electrolytes in Frozen Human Serum

This Standard Reference Material (SRM) is intended primarily for use in the calibration and standardization of procedures employed in clinical analysis for the determination of specific electrolytes in either diluted or undiluted human serum or plasma. This SRM can be used for standardizing direct-reading ion-selective electrode analyzers [1] and for validating working or secondary reference materials. A unit of SRM 956a consists of six flame-sealed ampoules of frozen human serum, two ampoules each of three different concentration levels. Each ampoule contains approximately 2.00 mL of human serum.

Certified Concentrations Values: The certified concentrations of the serum analytes and density, listed in Table 1, are the means of results based on measurements using a single primary method. All analyte concentrations are certified as totals. The primary method for potassium and total calcium is based on isotope dilution-thermal ionization mass spectrometry [2]. The primary method for the determination of sodium is based on gravimetry after ion exchange separation [3]. The primary method for magnesium and lithium is based on isotope dilution mass spectrometry using inductively coupled plasma mass spectrometry [4]. Density is determined by weighing calibrated pycnometers.

Reference Concentration Values: Reference concentration values for ionized calcium and chloride are provided in Table 3. The method of analysis for chloride is microcoulometry. The method of analysis for ionized calcium is ion selective electrode (ISE) potentiometry, the candidate designated comparison method (DCM) [5]. Ionized calcium data was obtained using at least six 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: This SRM is valid until the date printed on the exterior package label, within the measurement uncertainties specified, provided the SRM is handled and stored in accordance with the instructions given in this certificate. 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. Return of the attached registration card will facilitate notification.

The overall direction and coordination of the technical measurements leading to the certification of all electrolytes except ionized calcium and chloride were performed by R.L. Watters, Jr., of the NIST Chemical Science and Technology Laboratory. Coordination of the technical measurements leading to the value assignment of chloride was performed by F.R. Guenther of the NIST Analytical Chemistry Division. Coordination of analyses for value assignment of ionized calcium using the DCM was performed by G.N. Bowers, Jr., at Hartford Hospital, Hartford, CT.

The overall coordination of the NCCLS Subcommittee on Electrolytes' analyses for ionized calcium was performed by P. D'Orazio of Instrumentation Laboratory (formerly of Chiron Diagnostics).

The support aspects concerning the preparation, certification, issuance, and revision of this SRM were coordinated through the NIST Standard Reference Materials Program by J.C. Colbert and B.S. MacDonald of the NIST Measurement Services Division.

Willie E. May, Chief
Analytical Chemistry Division

John Rumble, Jr., Chief
Measurement Services Division

Gaithersburg, MD 20899
Certificate Issue Date: 10 July 2003
See Certificate Revision History on Page 4

Analytical measurements at NIST were performed by S.E. Long, M.S. Rearick, J.M. Smeller, and T.W. Vetter of the NIST Analytical Chemistry Division.

Statistical consultation and data evaluation for all electrolytes except ionized calcium and chloride was provided by L.M. Gill of the NIST Statistical Engineering Division. Statistical consultation for ionized calcium was provided by M.G. Vangel of the NIST Statistical Engineering Division. Statistical consultation and analysis for chloride was provided by W.F. Guthrie of the NIST Statistical Engineering Division.

The development of this SRM is the result of a cooperative research effort between NIST and the former NCCLS Subcommittee on Electrolytes. P. D'Orazio, Instrumentation Laboratory, and G. Graham, Johnson & Johnson Clinical Diagnostics (retired) are co-chairmen of the former NCCLS Subcommittee on Electrolytes.

NOTICE AND WARNING TO USERS

SRM 956a 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 [6].

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 °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 ALL ELECTROLYTES EXCEPT IONIZED CALCIUM

Place the ampoule to be used inside another container, such as a plastic test tube, 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, it should not be used. The serum in intact ampoules should be thawed, warmed to room temperature, and mixed by inverting gently at least five times before sampling.

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 carefully controlled conditions given below [5].

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 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 POOLS¹

SRM 956a was prepared by the Diagnostics Group, Bayer Corporation, Middletown, VA. The material SRM 956a was prepared from 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 °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 7.5 at 25 °C. Finally, 2.0 mL aliquots of each subpool were dispensed into 6.0 mL pre-scored cryule glass (33 Expansion Flint) ampoules flushed with an inert gas plus 5 % CO₂ overlay, flame sealed, and stored at -50 °C.

Analysis for Chloride: Value assignment of the concentrations of chloride was performed from measurements obtained over four analysis days on each of the three concentration levels. SRM 909, 909a, and 909b were used as controls. Chloride measurements were performed on a Labconco Chloridometer, on a low current range using conditions as prescribed by the manufacturer.

Analyses for Ionized Calcium in SRM 956a Using the Candidate DCM: For the determination of the concentrations of ionized calcium (iCa²⁺), a candidate DCM employing ion-selective electrode potentiometry was used. The procedure described in Appendix A of Reference 5 was carried out using at least six different calcium selective membranes shown to have acceptable iCa²⁺ recovery using an independent serum pool [5]. At least 20 ampoules per concentration level of the SRM were used. The iCa²⁺ concentrations are not corrected to pH 7.40, but are measured and reported at the actual pH values at the time of analysis. Based on the known relationship between pH and iCa²⁺ for SRM 956a, it is estimated that the ampoule-to-ampoule variability in iCa²⁺ due to pH variability alone is less than 0.005 mmol/L.

An Interlaboratory Study to Determine the Usefulness of SRM 956a as an Ionized Calcium Reference Material: An interlaboratory study was conducted involving 19 industrial and clinical laboratories with an interest in ionized calcium standardization [5]. The objectives of the multisite study were to determine if SRM 956a is compatible with the various types of commercial instrumentation available for measurement of ionized calcium, and if SRM 956a would be useful as a reference material to obtain uniformity among the various types of commercial instrumentation in the field. The list of participants is shown in Appendix A of this certificate. An effort was made to include at least one representative of all commercial instrumentation for the measurement of ionized calcium known to the subcommittee at the beginning of the study (April 1996). The interlaboratory study basically follows the recommendations given in ASTM E 691-92 [7].

Table 1. Certified Concentrations and Uncertainties^a for Electrolytes in SRM 956a

Electrolyte	Level I	Level II	Level III
Total Calcium (mmol/L)	3.025 ± 0.016	2.570 ± 0.016	2.127 ± 0.009
Lithium (mmol/L)	2.083 ± 0.005	1.334 ± 0.004	0.570 ± 0.002
Magnesium (mmol/L)	1.441 ± 0.003	0.947 ± 0.002	0.448 ± 0.001
Potassium (mmol/L)	6.008 ± 0.020	3.985 ± 0.020	2.025 ± 0.008
Sodium (mmol/L)	121.4 ± 0.3	141.0 ± 0.3	160.9 ± 0.4
Density (g/mL) at 22 °C	1.0242 ± 0.0004	1.0245 ± 0.0005	1.0251 ± 0.0004

^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 [8] 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, such as spike calibration. 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 956a 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 except chloride are listed in Table 2.

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

Electrolyte	Level I		Level II		Level III	
	u_c	v_{eff}	u_c	v_{eff}	u_c	v_{eff}
Total Calcium	0.0066	7	0.0063	5	0.0043	13
Lithium	0.0027	90	0.0018	41	0.00082	87
Magnesium	0.0013	26	0.00091	20	0.00043	34
Potassium	0.0090	12	0.0089	11	0.0038	11
Sodium	0.15	26	0.16	18	0.19	21

Table 3. Reference Concentrations (mmol/L) for Ionized Calcium and Chloride in SRM 956a

	Level I	Level II	Level III
Ionized Calcium ^a	1.741 ± 0.018	1.411 ± 0.017	1.091 ± 0.010
Chloride ^b	98.94 ± 0.10	113.79 ± 0.13	129.05 ± 0.14

^a The results are expressed as the reference value ± the expanded uncertainty. The expanded uncertainty is expressed as a 95 % confidence interval.

^b The reference values are the mean results of measurements made at NIST. The uncertainties in each reference value are expressed as expanded uncertainties, $U = k u_c$, calculated according to the methods described in the ISO Guide [8]. The quantity u_c represents, at the level of one standard deviation, the uncertainty in the mean concentration of chloride. The quantity k is a coverage factor used to obtain an expanded uncertainty with an approximate confidence level of 95 %. The value of the coverage factor, $k = 2.00$, is determined from the Student's t -distribution with 60 degrees of freedom and a confidence level of 95 %.

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).
- [2] Moore, L.J.; Machlan, L.A.; *High Accuracy Determination of Calcium in Blood Serum by Isotope Dilution Mass Spectroscopy*; Anal. Chem., Vol. 44, p. 2291 (1972).
- [3] Moody, J.R.; Vetter, T.W.; *Development of the Ion-Exchange-Gravimetric Method for Sodium in Serum as a Definitive Method*; J. Res. Natl. Inst. Stand. Technol., Vol. 101, pp. 155-164 (1996).
- [4] Bowers, G.N.; Fassett, J.D.; White V.E.; *Isotope Dilution Mass Spectrometry and the National Reference System*; Clin. Chem., Vol. 65, pp. 475R-479R (1993).
- [5] 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).
- [6] U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories*; U.S. Government Printing Office: Washington, DC (1988).
- [7] ASTM E 691-92, *Standard Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method*; Philadelphia, PA (1992).
- [8] *Guide to the Expression of Uncertainty in Measurement*, ISBN 92-67-10188-9, 1st Ed., ISO, 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).

<p>Certificate Revision History: 10 July 2003 (Appendix with reference values for glucose added); 19 November 2001 (This technical revision reports the addition of reference values for ionized calcium and chloride); 13 November 1996 (Original certificate date).</p>
--

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 the Internet <http://www.nist.gov/srm>.

APPENDIX A

The laboratories listed below participated in the interlaboratory study to determine the usefulness of SRM 956a as an ionized calcium reference material:

R. Byrd and G. Priest: AVL Scientific Corporation; USA
D. Pistone: Baxter Lytning Systems; USA
C. Sachs: CHU Necker-Enfants; Malades, France
P. D'Orazio: Ciba Corning Diag. Corporation; USA
S. Sena: Danbury Hospital; USA
J. Toffaleti: Duke University Medical Center; USA
A. Maas: Euro-Trol B.V.; The Netherlands
W. Schmehl: Fresenius AG; Germany
N. Peled: i-Stat Corporation; USA
A. Manzoni: Instrumentation Laboratory; USA
S. Mansouri: Mallinckrodt Sensor Systems; USA
M. Burritt: Mayo Clinic; USA
P. Makris: Medica Corporation; USA
N. Rehak: National Institutes of Health; USA
W. Lambert: NOVA Biomedical; USA
F. Zoppi: Ospedale Niguarda Ca'Granda; Italy
P. Black and J. Wandrup: Radiometer America; USA
L. Larsson: Univ. Of Linkoping; Sweden
T. Okorodudu: Univ. Texas Medical Branch; USA

APPENDIX B

Reference Concentrations for Glucose in SRM 956a

	mg/dL	mmol/L
All Levels	43.14 ± 0.45	2.395 ± 0.025

The reference concentration value was determined from measurements made at NIST using a method based on isotope dilution gas chromatography mass spectrometry. The method used involves spiking a known mass of serum with a known mass of glucose-¹³C₆. After isolation of the glucose from the serum, it is converted to a dibutylboronate acetate derivative for the GC/MS measurements. The instrument is calibrated using known mixtures of SRM 917b Glucose and the same glucose-¹³C₆ used to spike the serum samples.

The uncertainty in the reference concentration is calculated as $U = ku_c$. The quantity u_c is the combined standard uncertainty, calculated according to the ISO guide [10], and accounts for the combined effect of the components of uncertainty. The coverage factor, k , is determined from the Student's t -distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence level.

REFERENCE

- [1] White V.E.; Welch, M.J.; Sun, T.; Sniegoski, L.T.; Schaffer, R.; Hertz, H.S.; Cohen, A.; *The Accurate Determination of Serum Glucose by Isotope Dilution Mass Spectrometry – Two Methods; Biomed. Mass Spectrom.*, Vol. 9, pp.395-405 (1982).