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Certification of Standard Reference Material® 956e Electrolytes in Frozen Human Serum

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Lee L. Yu
Charles A. Barber*
Steven J. Christopher
Regina A. Easley
Monique E. Johnson
Karen E. Murphy†
Thomas W. Vetter
Jason F. Waters
Laura J. Wood†
Chemical Sciences Division
Material Measurement Laboratory

Dennis D. Leber
Statistical Engineering Division
Information Technology Laboratory

Stephen Chin
Murli Narayan
Michael Nash
Alexander Rhodes
R&D Current Product Support
Siemens Healthineers Point of Care

* *Former NIST employee; all work for this publication was done while at NIST.*
† *Retired*

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U.S. Department of Commerce
Howard Lutnick, Secretary

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Craig Burkhardt, Acting Under Secretary of Commerce for Standards and Technology and Acting NIST Director

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April 2025

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Author ORCID iDs

Yu LL	0000-0002-8043-6853
Barber CA	0000-0002-4968-7486
Christopher SJ	0000-0002-6605-5229
Easley RA	0000-0002-1876-2893
Johnson ME	0000-0001-5096-2003
Leber DD	0000-0003-4179-5586
Murphy KE	0000-0003-4115-6231
Vetter TW	0000-0003-1442-2530
Waters JF	0000-0002-9901-2188
Wood LJ	0000-0002-4294-7636

Contact Information
Please address technical questions you may have about this SRM to srms@nist.gov where they will be assigned to the appropriate Technical Project Leader responsible for support of this material. For sales and customer service inquiries, please contact srminfo@nist.gov

Abstract

The National Institute of Standards and Technology (NIST) Standard Reference Material® (SRM®) 956e Electrolytes in Frozen Human Serum is 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, and for validating methods used to measure electrolytes in similar matrices. The material was produced commercially in accordance with the protocol described in Clinical Laboratory Standards Institute (CLSI) Standard C29-A2, *Standardization of Sodium and Potassium Ion-Selective Electrode Systems to the Flame Photometric Reference Method*. This publication documents the production, analytical measurements, and statistical evaluations leading to the certification of the SRM.

Keywords

Electrolyte; Human serum; Standard Reference Material (SRM); Calcium (Ca); Ionized calcium (iCa); Chloride (Cl^-); Lithium (Li); Magnesium (Mg); Phosphorus (P); Potassium (K); Sodium (Na); Ion selective electrode.

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Purpose and Description

This Standard Reference Material® (SRM®) is 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. A unit of SRM 956e consists of six ampoules of frozen human serum with two ampoules at each of three mass concentration levels. Each ampoule contains nominally 2.0 mL of serum.

NIST is guided by and adheres to the ethical principles set forth in the Belmont Report: *Ethical Principles and Guidelines for the Protection of Human Subjects of Research*[*]. SRM 956e was developed after an appropriate human subjects' research determination that the work is *not human subjects research* as defined in Department of Commerce Regulations, 15 CFR 27, also known as the Common Rule (45 CFR 46, Subpart A), for the Protection of Human Subjects.

Warning: SRM 956e is a human-sourced material. Handle as a biohazardous material capable of transmitting infectious disease.

SRM 956e was prepared from human serum obtained from an FDA licensed blood collection facility. All units of serum used in the preparation of this product were tested negative or non-reactive for HBsAg, Anti-HIV I/II, Anti-HCV, HIV-1 RNA, HCV RNA, and syphilis according to FDA guidelines. 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.

Acknowledgments

The authors thank Dave Duewer of the NIST Chemical Sciences Division for assistance in the preparation of this report.

[*] Available through: https://www.hhs.gov/ohrp/regulations-and-policy/belmont-report/index.html

1. Introduction

The electrolyte balance between intracellular and extracellular environments is responsible for osmotic gradients which regulate the hydration of the human body, maintain blood pH, and control nerve and muscle function. Electrolyte balance is a critical biomarker, and the electrolyte levels must be maintained in the tight range for human health. Thousands of clinical electrolyte measurements are performed on a daily basis in the U.S. alone.

To provide primary traceability for these measurements, the National Institute of Standards and Technology (NIST) developed and maintains the Standard Reference Material® (SRM®) 956 Electrolytes in Human Serum series. These materials are used worldwide for the calibration (principally direct-reading ion-selective electrode analyzers) and validation of analytical instrumentation, procedures, and methods employed in clinical analysis for the determination of electrolytes in either diluted or undiluted human serum or plasma.

1.1. History of the SRM 956 Series

The initial member of the series, SRM 956 Electrolytes in Human Serum for Ion Selective Electrodes, was introduced in 1990 and delivered certified values for sodium and potassium [1]. SRM 956a Electrolytes in Frozen Human Serum was issued in 1996 and delivered certified values for lithium, sodium, magnesium, potassium, total calcium, and serum density as well as non-certified values for ionized calcium, chloride, and glucose [2]. SRM 956b Electrolytes in Frozen Human Serum was issued in 2004 and delivered certified values for lithium, sodium, magnesium, chloride, potassium, total calcium, and serum density as well as non-certified values for ionized calcium [3]. SRM 956c Electrolytes in Frozen Human Serum was issued in 2009 and delivered certified values for lithium, sodium, magnesium, chloride, potassium, and total calcium as well as non-certified values for ionized calcium and serum density [4]. SRM 956d Electrolytes in Frozen Human Serum was issued in 2015 and delivered certified values for lithium, sodium, magnesium, chloride, potassium, and total calcium as well as non-certified values for ionized calcium, phosphate, total phosphorus, and serum density [5]. SRM 956e Electrolytes in Frozen Human Serum was issued in late 2024 and delivers certified values for lithium, sodium, magnesium, chloride, potassium, and total calcium as well as non-certified values for ionized calcium, total phosphorus, and serum density [6].

To date, more than 4300 units of the SRM 956 series have been sold. About half of these sales have been to customers within the US. The countries and regions in which customers account for at least 2 % of the total sales are displayed in Fig. 1. The proportions of sales over time are displayed in Fig. 2.

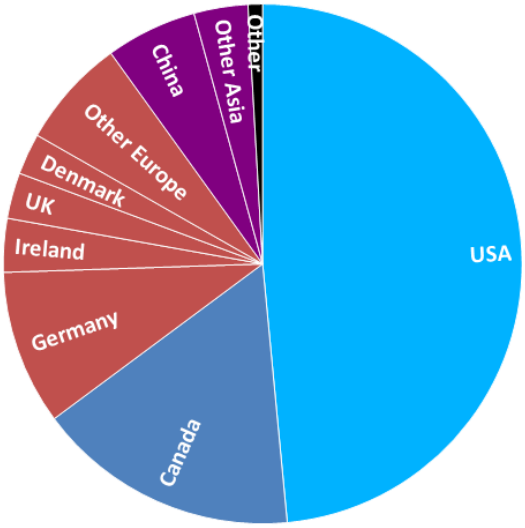

Fig. 1. Geographical Location of SRM 956 Series Customers.

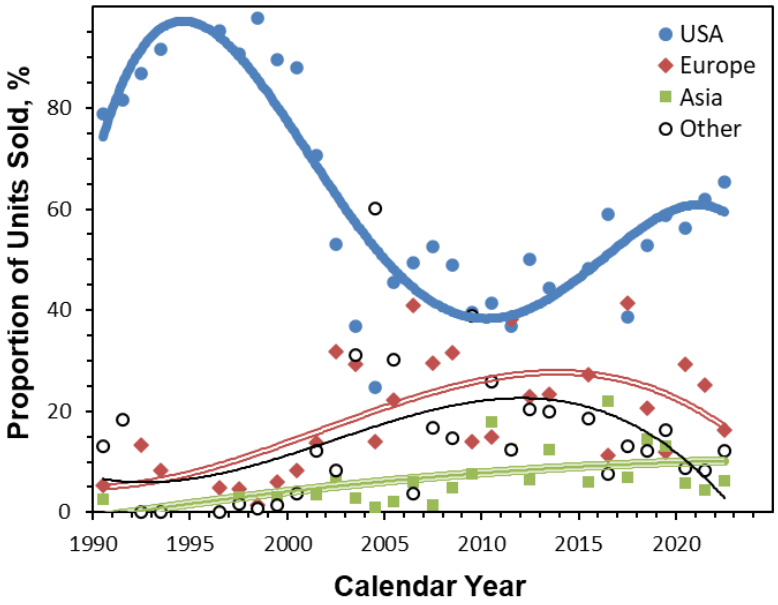

Fig. 2. Geographical Distribution of SRM 956 Series Sales Over Time.

The solid circles and the thick blue polynomial trendline display the proportion of sales to customers within the USA. Solid diamonds and the red double-line polynomial trendline display the proportion of units sold to customers in Europe (including the United Kingdom); solid squares and the triple-line green polynomial trendline display the proportion sold to customers in Asia. The open circles and thin black polynomial trendline display the proportion of units sold to customers elsewhere.

1.2. Value Assignments

Measurement results for serum density and the lithium (Li), sodium (Na), magnesium (Mg), phosphorous (P), chloride (Cl^-), potassium (K), and total calcium (Ca) content of the SRM 956e materials are based on measurements made by NIST. Measurement results for ionized calcium (iCa) are based on measurements made by Siemens Healthineers. All statistical analyses were performed at NIST.

The serum density, Li, Mg, Na, Cl^-, K, and Ca values are asserted to be traceable to the International System of Units (SI) through use of measurement process modes documented in [7]. The P and iCa results are considered reliable for use in quality control and method harmonization but are not considered traceable to the SI.

All the elemental results are stated both in units of mass concentration (mg/dL) and amount concentration (mmol/L).

The remainder of this document describes the SRM 956e materials and the various measurement methods used in their characterization.

2. Materials, Production, and Verification

A contract was awarded for the preparation of SRM 956e Electrolytes in Human Serum. The materials for SRM 956e were produced according to a NIST protocol that is consistent with the production procedure described in Clinical Laboratory Standards Institute (CLSI) Standard C29-A2 *Standardization of Sodium and Potassium Ion-Selective Electrode Systems to the Flame Photometric Reference Method* [8].

The production of candidate SRM 956e followed the NIST-designed protocol detailed in the Statement of Requirement (SOR) for the contract. The products of candidate SRM 956e were delivered on dry ice to the NIST Office of Reference Materials (ORM). The design, performance requirements, and manufacturing processes for the SRM materials are described in the following extracts from the SOR and contractor's report. The verification of the candidate SRM 956e materials is described in Section 2.3.

2.1. Material Preparation

At an FDA-licensed blood collection facility, units of human whole blood 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 resulting units of serum were then shipped frozen on dry ice to the Contractor for further processing and formulation.

The serum units were thawed, pooled, and filtered through an Avicel cellulose slurry under vacuum to remove fibrin. Gentamycin 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.

Appropriate amounts of ACS grade chloride (or sodium) salts were added to the Level I and Level III sub-pools to adjust the concentrations of sodium, potassium, calcium, magnesium, phosphorus, and lithium to the desired levels. The Level II sub-pool was prepared by gravimetrically combining equal amounts of the Level I and Level III sub-pools, then adjusted with the appropriate salts as needed. The pH was adjusted to 7.4 at 37 °C using 5 % CO_2 in nitrogen.

Finally, 2.0 mL aliquots of each sub-pool were dispensed into prelabeled Wheaton unscored glass ampoules (part number W651469) flushed with nitrogen plus 5 % CO_2 overlay, flame sealed, and stored at –70 °C. The manufactured units were shipped to NIST and stored at the ORM facility in an -80 °C freezer.

2.2. Certificate of Test of Human Serum

A Certificate of Analysis issued by an FDA approved blood collection facility for the serum used to produce SRM 956e is shown in Fig. 3.

CERTIFICATE OF ANALYSIS

Product Description:	Human Serum – Off Clot
Catalog Number:	HS-1004
Lot Number:	BMS210703
Source:	Human Serum Off Clot
Filtration	0.2 micron filtered
Storage	< -15° C
Source	Human Donors
Preservative	None
Assay	**Results**
Appearance	Clear, straw yellow solution
Fibrinogen	Negative
pH	7.2
Total Protein	6.5 g/dL
Bioburden	< 10 CFUs/mL

All units contained in this product were Negative or non-reactive for tests for HBsAg, Anti-HIV I/II, Anti-HCV, HIV-1 RNA, and HCV RNA and syphilis screening according to FDA guidelines.

Fig. 3. Certificate of Test of Human Serum.

2.3. Contractor's Analysis Values

Lithium (Li), sodium (Na), magnesium (Mg), phosphate ($[PO_4]^{3-}$), chloride (Cl^-), potassium (K), calcium (Ca), and pH in SRM 956e material were measured at the Contractor's site and reported to NIST as part of the deliverables required by the SOR. Locally made quality assurance materials were used for quality assurance in the measurement of each analyte. The data from the Contractor were used to assess whether the material was manufactured correctly to the contractual specifications, such as analyte concentration level and homogeneity, but are not used for the assignment of certified values.

Measurements of Na, Mg, $[PO_4]^{3-}$, Cl^-, K, and Ca were made with Roche Cobas c501 clinical analyzer. Roche calibration modules were used for instrument calibration. Measurement of Li was made with an Abbott Architect c4000 clinical analyzer. An Abbott Lithium Calibrator was used for instrument calibration. Measurement of pH was made with a pH meter.

For each level, six randomly selected ampoules, including the first and the last from the production sequence, were used for testing the electrolytes and pH, except for chloride. Twelve randomly selected ampoules from the production sequence were used for the measurement of chloride. Duplicate samples from each ampoule were measured for electrolytes. One sample from each ampoule was measured for pH because the amount of serum in the ampoule was only enough to provide one measurement.

Table 1, Table 2, and Table 3 list the Contractor's measurement results for Levels 1, 2, and 3.

Table 1. Contractor's Measurement Results for SRM 956e Level 1.

Sample	Li mmol/L	Na mmol/L	Mg mg/dL	Phosphate mg/dL	Cl^- mmol/L	K mmol/L	Ca mg/dL	pH
1a	1.984	121	3.63	6.0	92	6.1	12.0	7.36
1b	1.976	122	3.60	6.1	94	6.1	12.1	
2a	1.965	122	3.58	6.1	95	6.1	12.0	7.35
2b	1.989	121	3.57	6.0	94	6.1	12.0	
3a	1.998	122	3.62	6.1	94	6.1	12.0	7.41
3b	1.995	122	3.54	6.1	95	6.0	12.1	
4a	1.984	121	3.58	6.1	95	6.1	12.1	7.37
4b	1.994	121	3.58	6.0	95	6.0	12.2	
5a	1.979	122	3.57	6.1	94	6.1	12.0	7.36
5b	2.003	121	3.60	6.1	95	6.1	12.1	
6a	1.977	121	3.58	6.1	95	6.0	12.1	7.40
6b	1.981	122	3.59	6.1	95	6.1	12.1	
Mean	1.985	121.50	3.587	6.075	94.33	6.075	12.067	7.375
SD	0.011	0.52	0.024	0.045	0.70	0.045	0.065	0.024
Target	2.0 ± 0.1	120 ± 3	3.6 ± 0.2	6.2 ± 0.3	110 ± 20	6.0 ± 0.2	12.0 ± 0.8	7.40 ± 0.05

Table 2. Contractor's Measurement Results for SRM 956e Level 2.

Sample	Li mmol/L	Na mmol/L	Mg mg/dL	Phosphate mg/dL	Cl^- mmol/L	K mmol/L	Ca mg/dL	pH
1a	1.288	140	2.42	4.6	108	4.1	10.1	7.35
1b	1.267	140	2.39	4.6	108	4.1	10.0	
2a	1.228	140	2.36	4.6	108	4.1	10.1	7.41
2b	1.275	141	2.37	4.6	108	4.0	10.1	
3a	1.283	140	2.40	4.6	109	4.2	10.1	7.43
3b	1.257	141	2.39	4.6	107	4.1	10.1	
4a	1.285	141	2.38	4.6	107	4.1	10.0	7.39
4b	1.271	142	2.41	4.6	108	4.1	10.1	
5a	1.284	140	2.38	4.6	107	4.1	10.2	7.41
5b	1.263	141	2.40	4.6	107	4.1	10.1	
6a	1.288	139	2.39	4.6	108	4.0	10.0	7.43
6b	1.275	140	2.41	4.6	107	4.1	10.1	
7a	1.288				108			
7b	1.294				108			
8a	1.286				109			
8b	1.284				109			
9a	1.297				108			
9b	1.274				107			
10a	1.275				107			
10b	1.277				108			
11a	1.298				108			
11b	1.271				108			
12a	1.267				108			
12b	1.288				108			
Mean	1.278	140.42	2.392	4.600	107.83	4.092	10.083	7.403
SD	0.015	0.79	0.017	0.000	0.64	0.051	0.058	0.030
Target	1.25 ± 0.1	140 ± 3	2.4 ± 0.2	4.6 ± 0.3	110 ± 20	4.0 ± 0.2	10.0 ± 0.8	7.40 ± 0.05

Table 3. Contractor's Measurement Results for SRM 956e Level 3.

Sample	Li mmol/L	Na mmol/L	Mg mg/dL	Phosphate mg/dL	Cl^- mmol/L	K mmol/L	Ca mg/dL	pH
1a	0.534	160	1.29	3.3	123	2.2	8.0	7.36
1b	0.534	161	1.30	3.4	123	2.2	8.0	
2a	0.534	160	1.29	3.4	124	2.2	8.2	7.38
2b	0.548	161	1.31	3.4	123	2.2	8.0	
3a	0.545	160	1.30	3.4	123	2.2	8.0	7.4
3b	0.542	161	1.27	3.3	124	2.1	8.0	
4a	0.542	161	1.30	3.3	123	2.2	8.1	7.44
4b	0.524	161	1.31	3.4	123	2.2	8.2	
5a	0.528	161	1.29	3.4	123	2.2	8.1	7.4
5b	0.550	160	1.30	3.4	124	2.2	8.0	
6a	0.549	159	1.32	3.3	123	2.2	8.0	7.43
6b	0.516	160	1.30	3.4	122	2.2	8.0	
7a	0.545				123			
7b	0.543				124			
8a	0.527				124			
8b	0.517				123			
9a	0.559				123			
9b	0.539				124			
10a	0.556				124			
10b	0.562				124			
11a	0.541				123			
11b	0.541				124			
12a	0.547				123			
12b	0.556				124			
Mean	0.541	160.42	1.298	3.367	123.38	2.192	8.1	7.402
SD	0.012	0.67	0.013	0.049	0.58	0.029	0.080	0.030
Target	0.5 ± 0.1	160 ± 3	1.2 ± 0.2	3.1 ± 0.3	110 ± 20	2.0 ± 0.2	8.0 ± 0.8	7.40 ± 0.05

In the initial set of six ampoules, the Li values in Levels 2 and 3 failed an analysis of variance (ANOVA) test for homogeneity at a 95 % level of confidence. Twelve ampoules each at Level 2 and Level 3 were used to retest Li in these two levels. The ampoules were randomly selected to include the first and the last ampoules from the remaining stock of ampoules that were stored in the order of the production sequence. No inhomogeneity for the element was detected in this second test.

The SOR specifies that the relative standard deviation (RSD) for measurement replications be within 1 *%* so that the expanded uncertainty of the certified values can be kept to a minimum, which is typically around 1 % relative. Analytes in all levels met this criterion except for Li in Level 3. There are variations in the accuracy that are routinely achievable for different elements in SRM 956, and the 1 % RSD is a catch-all criterion. This criterion is harder to achieve for Li in level 3 as indicated by the relative expanded uncertainty at 1.4 %, 1.5 %, and 2.6 % for the b, c, and d iterations of the SRM, respectively. In comparison, the relative expanded uncertainty of the contractor's measurement due to replication is 0.96 %. Given sample replication is the primary source of the measurement uncertainty in Li measurement, the homogeneity indicated by the contractor's measurement fits the purpose of SRM 956e's intended use.

2.4. Material Homogeneity

The human serum is believed to be homogeneous for the analytes of interest in this reference material. This belief is supported by the results of the homogeneity analysis performed by the Contractor using clinical analyzers, where it was concluded that no detectable heterogeneity was observed for any of the analytes. Given this belief and the supporting analysis, the uncertainties in the measurands described elsewhere in this document need not be expanded to include vial-to-vial variability. The analytes in SRM 956e are projected to be stable for at least ten years post-production based on the experience with the 956 series of SRMs produced in conformity with CLSI C29A2 standard [8]. As part of quality assurance, NIST will monitor the stability of the analytes throughout the period of validity the SRM.

3. Serum Density

Density values are required to allow conversion of values determined in serum solutions from a mass fraction basis (e.g., mg/kg) to mass concentration (e.g., mg/dL) and amount concentration (e.g., mmol/L) basis.

Density was determined in test portions of the three levels of SRM 956e. Ampoules of SRM 956e were removed from storage at -80 °C, allowed to thaw and equilibrate to a measured ambient temperature, and gently mixed. Ampoules were scored with a file. Serum was withdrawn from opened ampoules and vials into a 1000 µL Lang-Levy micropipette whose volume had been calibrated with water and whose empty mass had been measured. The pipette was wiped with a Kimwipe and placed on a weighing cradle in front of a static-eliminating source for a few seconds. The pipette and cradle were transferred to an analytical balance with an internal static-elimination device and their mass was determined. The density was calculated at the ambient temperature by dividing the measured mass of the serum by the calibrated pipette volume. The density at the ambient temperature was corrected to the density at 22 °C.

Nine test portions of Level 1 and ten test portions each of Levels 2 and 3 were determined.

3.1. Materials

Ten ampoules for each of the three SRM 956e Levels were selected for analysis.

Reagents used included concentrated sulfuric acid (95 % to 98 % mass fraction) from Mallinkrodt and 100 % mass fraction ethanol from the NIST stockroom. All water used was collected from an in-house 18 MΩ·cm water source.

A 70 % (by volume) ethanol solution was prepared from the 100 % ethanol and water. According to the Centers for Disease Control and Prevention, for alcohols the “cidal activity drops sharply when diluted below 50% concentration, and the optimum bactericidal concentration is 60%–90% solutions in water (volume/volume)” [9].

3.2. Procedure

3.2.1. Initial Preparation of Lang-Levy Micropipettes

Four 1000 µL pipettes were chosen for the analysis. Using four micropipettes, rather than just one or two allowed for there to be sufficient time for a pipette to dry and equilibrate to ambient temperature prior to its repeated use.

Each pipette was rinsed several times with concentrated sulfuric acid to remove any adhering organics. Residual acid was rinsed out with water, followed by ethanol, and the pipette was dried by attaching a tube connected to a vacuum flask connected to house vacuum. After the dried pipette was disconnected from the vacuum flask tube, it was viewed in good lighting to make sure that there was no residual solution in the pipette.

3.2.2. Mass Measurements

None of the recorded masses are corrected for air buoyancy because, with similar densities for the water and serum, the buoyancy correction in the calculation of the serum density buoyancy correction is insignificant. All masses are calculated based on the mean of two measurements with each measurement calculated as the difference between the recorded mass and the recorded tare. All masses used in the calculations were determined on an analytical balance to a resolution of 10 µg.

3.2.3. General Calibration and Density Determination Procedure Structure

The density of each level of serum was determined over a single analysis day. Initially, the mass of water contained within each of the four 1000 µL pipettes was measured in duplicate to determine a calibrated volume. The calibrated volume for each of the four 1000 µL pipettes was calculated based on the density of water at the ambient temperature. Although, for the measurements made for this analysis, the change in the volume of water as a function of temperature is relatively significant within the measured temperature ranges, the dimensional volume of the glass pipette does not change significantly over the same temperature range. Thus, for the purposes of these measurements, the pipette volume at ambient temperature is the same as the pipette volume at 22 °C. Temperature was monitored using a meter and thermocouple of known accuracy. Water density values are based on the density of air-saturated water from Jones and Harris [10].

The calibration with water was followed by determining the mass of nine or ten test portions of SRM 956e and finally a repeat of the duplicate measurement of the mass of water contained within each of the four 1000 µL pipettes to determine a calibrated volume.

The calibrated volume of each 1000 µL pipette used to determine the density of the serum was calculated from the measurements taken prior to and after the measurements of the serum as the mean of four measurements. The pipette was always handled with gloved hands to minimize transfer of heat and residue. The mass of the pipette and its weighing cradle were measured by placing the cradle diagonally across the balance pan. Once the suction device was attached firmly to the pipette, the pipette was grasped using the suction device to minimize transfer of heat to the pipette. For sucking the solution into the pipette, the fingers of one hand were used to rotate the thumbscrew to suck up solution. Fingers of the other hand were pressed on the black elastic attached to the pipette to maximize the suction process.

Since an anti-static device was used outside the balance and one was incorporated into the balance, a wipe with a damp cloth to reduce static was not needed. Kimwipes were used to wipe external residual solution off the pipette tip. An advantage of using Kimwipes was that they could be disposed of easily as biohazardous waste when they were used to wipe the pipettes containing serum.

3.2.4. Calibration Procedure for the 1000 µL Pipettes

Each dry pipette was allowed to equilibrate to ambient temperature for at least 20 minutes after it had been dried with ethanol prior to any mass measurements of the empty pipette. The empty pipette was placed on a weighing cradle in front of a static-eliminating source for a few

seconds. The pipette and support were placed on the balance pan (holding the cradle only) at an angle to avoid having the pipette touch the interior balance walls or doors. After the balance door was closed, the mass of the pipette on the cradle was determined 25 s later. The pipette and cradle were removed from the balance, placed in front of the static-eliminating source, and the balance doors were closed. The balance tare was determined 25 s later. The mass of the pipette and cradle and the balance tare were determined again so that duplicate measurements of the pipette and cradle net mass (measured pipette and cradle mass minus tare mass) were made.

A thumbscrew suction device was attached to the pipette, and the pipette was inserted upright into a beaker of water next to the balance. The water had been poured into the beaker from a bottle that had been filled with water at least one day prior and was placed next to the balance at least 30 minutes prior to sampling. This time interval helped to ensure equilibrium of the water temperature with the air temperature that was measured. The pipette was filled close to its constriction and a final filling to the constriction was monitored by looking through a magnifying glass. Once it had been filled to the constriction, the pipette was vertically lifted out of the water. Once it was removed from the water the pipette was oriented horizontally and the thumbscrew was rotated three times to pull air into the tip. The area around the pipette tip was wiped twice with a Kimwipe to remove external residual solution, the suction device was removed, and the pipette was placed on the cradle in front of a static-eliminating source for a few seconds. The pipette and cradle were then transferred to the balance and their mass was determined in duplicate in the same manner as was done for the empty pipette.

3.2.5. Preparation of the Pipette for Subsequent Analysis After Measurement of the Contained Water Mass

The water in the pipette was removed using a pipette pump in reverse. Then the end of the pipette was connected to a hose attached to a vacuum flask, vacuum was applied, and the tip of the pipette was briefly dipped into 100 % ethanol. To enhance purging, the pipette was held vertically, with the tip up, for about 5 s. The dipping and purging step was repeated three times with the tip remaining in ethanol for a few seconds on the last dip, followed by holding the tip up to purge for about 10 s until residual ethanol in the tip appeared to have evaporated. The pipette under vacuum was placed horizontally for at least 5 min before vacuum was disconnected. The dried pipette was checked to make sure that it did not contain any residual solution and was allowed to equilibrate to ambient temperature for at least 20 min. The calibration of another pipette was initiated during the extended vacuum purge and at least one more pipette could be calibrated and purged during the equilibration time.

3.2.6. Optimized Thawing Schedule

At the beginning of each analysis day, ten SRM 956e ampoules of one level were removed from -80 °C storage. Three ampoules were transferred to a biohazard hood to thaw while seven were placed in a nearby freezer at nominally 20 °C. While the samples were thawing, the calibration of the pipettes was performed. After at least 90 min had transpired, the contents of the vials and ampoules were gently mixed by inverting at least 6 times. The tops of the vials and

ampoules were tapped to encourage any solution in the top to drop into the solution below. All subsequent samples were treated in the same manner.

The ampoules held at 20 °C were removed, three or four at a time, over the course of the analysis day. This two-step process efficiently minimized the time serum samples spent at ambient temperature.

Only nine ampoules of SRM 956e Level 1 were analyzed because one of the ampoules leaked once it had thawed. Ten ampoules of both Levels 2 and 3 were analyzed.

3.2.7. Extraction Procedure Used for Serum

The procedure for the measurement of the mass of the empty and full pipette for the serum was the same as the procedure described for the water in Section 3.2.4. The procedure for filling the pipette with serum and purging the contents of the pipette after measurement was different for the serum, compared to the water calibration, because of the need to treat the serum as a biohazard.

The ampoule or vial was opened at least 30 min after it had been mixed. Once it had been opened, serum withdrawal was started immediately to minimize evaporation. The bulk of the serum was withdrawn within 30 s or less and sampling was complete within about 1 min. The pipette was filled with serum directly from the vial or ampoule in the biohazard hood, rather than next to the balance. The ambient temperature was monitored both inside the hood and next to the balance; however, only the temperature inside the hood was used as an indication of the ambient serum temperature.

After measurement, the serum in the pipette was purged into a container using a pipette pump in reverse. The pipette tip was then held over another container and, using a squirt bottle with a disposable pipette tip attached, water was rinsed into the opposite end and gently purged into the container using a pipette pump in reverse. The outside tip of the pipette was also rinsed with water a few times. Rinsing and purging was repeated four times to maximize the possibility that no serum remained in the pipette for subsequent rinses with alcohol. Residual serum can form a precipitate when it reacts with alcohol and thus change the internal volume of the pipette. To sanitize the pipette, five rinses with 70 % alcohol followed the water rinse and were done in a similar manner as the water rinse. To remove all remaining water, two rinses with 100 % ethanol were done in a similar manner to both the inside and outside of the pipette. Once these sets of rinses were complete, the pipette was connected to a vacuum flask and treated in the same manner as was done after the calibrations with water.

3.2.8. Equations Used to Calculate Pipette Volume and Serum Density

The mass of the water in the 1000 µL micropipette, m_w, was calculated:

$$\boldsymbol{m_w = m_{wF} - m_{wE}} \quad (1)$$

where m_{wF} is the mass of the micropipette full of water and m_{wE} is its mass empty.

The calibrated volume of the 1000 µL micropipette, V, was calculated:

$$\boldsymbol{V = m_{wF}/\rho_{amb}} \quad (2)$$

where ρ_{amb} is the density of water at the measured ambient temperature.

The mass of the serum in the 1000 µL micropipette, m_s, was calculated:

$$\boldsymbol{m_s = m_{sF} - m_{sE}} \quad (3)$$

where m_{sF} is the mass of the micropipette full of serum and m_{sE} is its mass empty.

The density of the serum, ρ_s, is calculated:

$$\boldsymbol{\rho_s = \frac{m_s}{V} q} \quad (4)$$

where q is used to correct the density at the ambient temperature to the density at 22.0 °C. The correction factor is calculated:

$$\boldsymbol{q = \frac{\rho_{22}}{\rho_{amb}}} \quad (5)$$

where ρ_{22} is the density of water at 22 °C. The correction factor for the serum can be based on the values for the density of water because the volumetric expansion of serum does not differ significantly from the value given for water [11].

3.3. Measurement Results

Table 4 lists the measured serum densities for SRM 956e Levels 1 to 3.

Table 4. Serum Density Measurements for SRM 956e.

	Serum Density, g/mL		
	Level 1	Level 2	Level 3
1	1.021 777	1.022 376	1.023 143
2	1.022 071	1.022 563	1.023 094
3	1.022 056	1.022 504	1.023 071
4	1.022 179	1.022 404	1.022 909
5	1.022 108	1.022 497	1.023 015
6	1.022 819	1.022 365	1.022 876
7	1.022 020	1.022 298	1.022 882
8	1.022 132	1.022 457	1.023 097
9	1.022 192	1.022 218	1.023 020
10		1.022 545	1.022 908
N:	9	10	10
Mean:	1.022 151	1.022 423	1.023 001
Standard Deviation:	0.000 279	0.000 111	0.000 100

The densities are displayed as a function of their within-Level measurement sequence in Fig. 4. The Level 1 materials were evaluated first and Level 3 last. Measurements for each Level were completed in a single day. The regular increase in mean density is a consequence of the Level 2 material being a blend of the Level 1 and Level 3 materials. The increase in measurement precision of the Level 2 and Level 3 materials relative to Level 1 is believed to be a consequence of increased analyst experience. The initial inexperience in performing the Level 1 measurements is a likely reason for the relatively higher value for Level 1 ampoule 6.

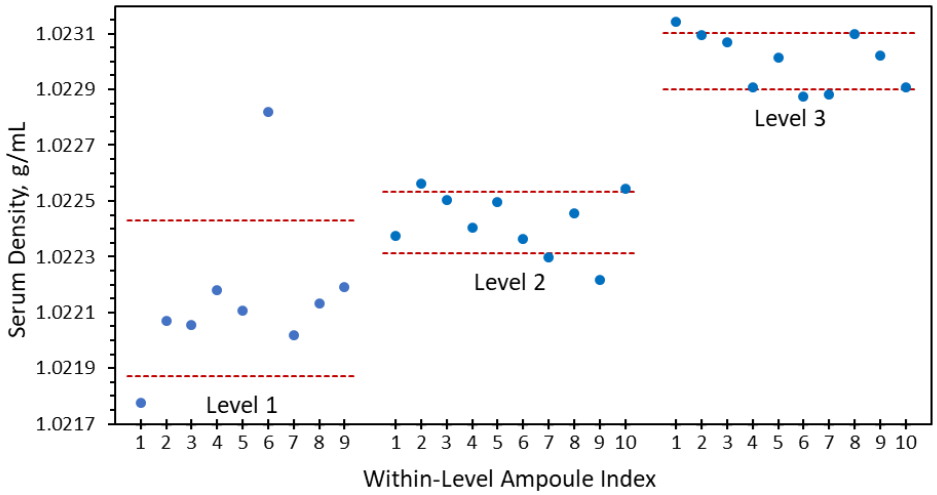

Fig. 4. Serum Density Measurements as a Function of Measurement Sequence.

Each symbol represents the measured serum density in one ampoule of SRM 956e. Dashed horizontal lines bound the mean ± standard deviation interval for the measurements at each Level.

3.4. Uncertainty Evaluation

3.4.1. Measurement Functions for Calculating Uncertainties

The measurement function used to calculate the uncertainty, based on Eqs. 1 to 6, is:

$$\boldsymbol{\rho_s} = \frac{\boldsymbol{m_s}}{\boldsymbol{qV}}\left(\frac{\boldsymbol{RW_T S_T}}{\boldsymbol{W_m}}\right) \tag{6}$$

where m_s, V, and q are defined as previously in Eqs. 2 to 4, and R, W_T, S_T, and W_m are placeholder quantities used to calculate additional sources of uncertainty: R is replication uncertainty of the calculated serum density, W_T is the water calibration temperature uncertainty, S_T is the serum temperature uncertainty, and W_m is the uncertainty of the mass of the water used to calibrate the pipette volume. The placeholder quantities are equal to 1 and the magnitude of the uncertainty is calculated on a relative fractional basis. Thus, these uncertainties do not influence the calculated value and each has units of dimension one.

The influence of the measured temperature on the calculated density is based on the table used for calculations of volumes and conversion to the density at 22 °C [10] but is not a direct input to the measurement function. The direct input of the mass of water used to calibrate the pipette volume is captured in the calculation of the pipette volume and its indirect input is the uncertainty of the mass measurement.

For each of the three levels of SRM 956e, the uncertainty of a specific test portion, selected because its density is similar to the mean density value, was calculated using a Kragten spreadsheet [12,13]. To estimate the standard uncertainties for the mean serum density, the calculated uncertainty is multiplied by the ratio of the mean serum density to the serum density for the specific test portion.

Uncertainties based on evaluation by Type A methods for replication are estimated from the standard deviation of the determined value and, for the measurement replication of the calibration of the pipette volume, are estimated from the pooled standard deviation for all four pipettes.

Uncertainties based on evaluation by Type B methods in the determination of the density have been assessed for the following procedural components of the determination: serum mass measurement, the temperature of the serum in the measurement of its mass for the determination of the density, water mass measurement, the temperature of the water in the measurement of its mass for the calibration of the pipette, and the uncertainty of the value used to convert to a density at 22 °C.

In general, all uncertainties are evaluated and calculated based on published information and previous work. The following steps of the procedure, and their possible effect on the measurement results, will be discussed: replication of the calculated serum density, general mass measurement, serum mass measurement, serum temperature, water mass, water temperature, replication of the calibration volume, and the conversion factor to calculate the density at 22 °C. The basis for the uncertainty evaluation of these components are summarized in Table 3 and are discussed in more detail below.

3.4.1.1. Replication of the Calculated Serum Density

The standard uncertainty is calculated as the standard deviation of the mean (aka “standard error”), which is the standard deviation of the determinations divided by the square root of the number of determinations.

3.4.1.2. General Mass Measurement

The balance has specifications for resolution, reproducibility, eccentricity, linearity, and span while the calibration certificate lists values for repeatability, eccentricity, and error of indication both for the balance “As Found” and “As Left”. The measurement function for “As Left” was used. The measurement uncertainty stated in the calibration certificate is likely to be greater than the actual balance uncertainty because: 1) the measurement replication is already accounted for, 2) eccentricity (dependent on the location of the mass on the pan) was minimized by consistently placing the weighing cradle diagonally across the center of the pan, and 3) determined values are nominally based on the mass of serum relative to the mass of water, so that biases in determining their mass may cancel out. However, the measurement function in the calibration certificate is used for lack of a better alternative:

$$\boldsymbol{U = (0.030 + m \times 0.0088)\ \mathrm{mg}} \tag{7}$$

where *U* is the 95 % level of confidence expanded uncertainty and *m* is the measured mass, in g. The standard uncertainty (*u*) is calculated by dividing *U* by 2, with the assumption that the expansion factor (*k*) is equal to 2.

3.4.1.3. Serum Mass Measurement

The serum mass measurement is calculated from the difference of the pipette containing serum and the empty pipette. This difference is used as the value of *m* in Eq. 7. Since the value is calculated based on two mass measurements, the uncertainty estimate is multiplied by $\sqrt{2}$ and then divided by 2 to calculate the standard uncertainty.

3.4.1.4. Serum Temperature

The uncertainty of the serum mass temperature is estimated based on the largest difference in temperature between subsequent serum mass measurements. At nominally 22°C, a temperature difference of 0.1 °C will have a relative difference of 0.0023 % on the calculated density [10]. Thus, the relative difference of 0.0023 % is multiplied by the difference divided by 0.1°C. Since the temperature difference is more likely to have a central tendency, the distribution is modeled as a triangular distribution.

3.4.1.5. Water Temperature

The uncertainty of the water mass temperature is estimated based on the largest difference in temperature between subsequent water mass measurements. The standard uncertainty is calculated in the same manner as the estimate of the uncertainty for the serum mass temperature.

3.4.1.6. Water Mass Measurement

The water mass measurement is calculated from the difference of the pipette containing water and the empty pipette. The standard uncertainty is calculated in a similar manner as the estimate of the uncertainty for the serum mass measurement, except as follows. The value for the difference is obtained from the mean difference for the number of micropipettes used.

3.4.1.7. Replication of the Calibration Volume

For each of the four micropipettes, a mean calibrated volume is calculated. The uncertainty of the calibrated volume is estimated as the pooled standard deviation of the four micropipettes. The pooled standard deviation is divided by the square root of the four measurements for each pipette to calculate the standard uncertainty. The pooled standard deviation, determined from four replicates of four pipettes, has 12 degrees of freedom.

A review of the two volumes calculated based on water masses measured prior to the serum mass measurements and after the serum mass measurements indicated that the four values sometimes had more of a bimodal, rather than a normal distribution. Modeling the uncertainty using the range of the four values with a triangular distribution results in calculated standard uncertainties with nominally similar values as those calculated using the pooled standard deviation. The similarity in the values of the two estimates led to using the pooled standard deviation, rather than the range, modeled as a triangular distribution, particularly because the bimodal distribution was only present sometimes, and ruling out a normal distribution based on only four measurements is difficult.

3.4.1.8. Conversion to Density at 22 °C

The uncertainty of the conversion to the density at 22 °C is based on the resolution of the table of water densities. At nominally 22°C, a temperature difference of 0.1 °C will have a relative difference of 0.0023 % on the calculated density [10]. Since the conversion factor is calculated as the ratio of two values, the relative difference is multiplied by $\sqrt{2}$. Since the difference is more likely to have a central tendency, the distribution is modeled as a triangular distribution.

3.4.1.9. Comments on Uncertainty Sources

Table 5 describes uncertainty components.

Table 5. Components of Uncertainty and Basis for Their Evaluation.

Input	Symbol	Uncertainty source	Unit	Basis	Distribution	Normalization
Serum density replication	R	Relative replication of mean for n ampoules	1	Standard deviation divided by sqrt(n)	normal	divide by √n
Serum mass	m	Equation from balance certificate	g	Eq. 7 where m is measured mass in g	U, k = 2	Divide by 2
Serum temperature	S_T	Relative temperature difference between subsequent serum measurements	1	Largest relative difference in density from table between temperatures	Triangular	Divide by √6
Water mass used to calculate pipette volume	W_m	Equation from balance certificate	1	Eq. 7 where m is measured mass in g, divided by mass (relative)	U, k = 2	Divide by 2
Water calibration temperature	W_T	Relative temperature difference between subsequent water measurements	1	Largest relative difference in density from table between temperatures	Triangular	Divide by √6
Calibration volume	C	Replication of mean for n measurements	mL	Pooled standard deviation	Normal	Divide by √n
Conversion to density at 22 °C	q	Relative uncertainty of 0.1 °C difference	1	relative difference in density for 0.1 °C change from 22.0 °C [10]	Triangular	Divide by √6

For level 1, the largest contribution was from the calibration volume (42 %), followed by the water temperature (18 %) and serum density measurement (16 %). As the ability to measure the calibration volume and serum density improved for levels 2 and 3, the uncertainty evaluated by Type A methods was reduced, which led to lower relative expanded uncertainties and different relative contributions to the variance. For level 2, the largest contribution is from the water temperature (39 %), followed by the calibration volume (19 %) and the serum temperature (18 %). For level 3, four of the components had nominally equal contributions of 20 % each. The uncertainty of the conversion of the density to 22 °C consistently had the lowest relative contribution.

The changing pattern of contributions to the variance demonstrate how improving the ability to pipette the water and serum in a consistent manner is a major factor in reducing the uncertainty.

3.5. Results

Table 6 summarizes the serum density measurement results for SRM 956e Levels 1 to 3. These results are based on gravimetry, a primary method, with consideration of all known sources of variability and bias [7]. They are traceable to the SI.

Table 6. Serum Density Summary Results for SRM 956e.

	Serum Density, g/mL		
Parameter	Level 1	Level 2	Level 3
Determined Value:	1.022 151	1.022 423	1.023 001
Type A uncertainty components:	0.000 088	0.000 044	0.000 034
Type B uncertainty components:	0.000 074	0.000 081	0.000 054
Standard uncertainty, u:	0.000 115	0.000 092	0.000 064
Expanded Uncertainty, U:	0.000 230	0.000 181	0.000 126
Relative Expanded Uncertainty, U_{rel}:	0.023 %	0.018 %	0.012 %

The small relative expanded uncertainties are negligible in all calculations involving density of the SRM 956e materials.

4. Value Assignment Calculations

Because the ampoules are deemed homogenous (Section 2.4), each independently prepared replicate, whether from the same or different ampoules, is viewed as an independent observation.

4.1. Analyte Evaluated Using a Single Method

For the six analytes (Li, Mg, P, Cl^-, K, and total Ca) that were evaluated using just one measurement method, values are assigned as the arithmetic means of the individual measurements. The associated uncertainty components, standard uncertainty (u), expanded uncertainty (U), and effective degrees of freedom (v) are evaluated for each analyte. The expansion factor, k, used in computing the expanded uncertainties, $U = ku$, is the 97.5th quantile of a Student's t distribution with the provided effective degrees of freedom. This expansion factor corresponds to an approximate 95 % confidence level.

4.2. Analyte Evaluated Using Multiple Methods

For two analytes (Na and ionized calcium), an estimated consensus value (μ), between-method effect (τ_i), and measurement error (ε_{ij}) were obtained using a Gaussian mixed-effects model with possible heterogeneous within-method variances [14,15,16].

$$\begin{aligned} &\boldsymbol{y_{ij}} \;=\; \boldsymbol{\mu + \tau_i + \varepsilon_{ij}} \begin{cases} \boldsymbol{i = 1, \ldots, m} \\ \boldsymbol{j = 1, \ldots, n_i} \end{cases} \\ &\textbf{Method}\colon \boldsymbol{\tau_i \sim N(0, \sigma_\tau^2)} \\ &\textbf{Error}\colon \boldsymbol{\varepsilon_{ij} \sim N(0, \sigma_\varepsilon^2)} \end{aligned} \tag{8}$$

where y_{ij} is the j^{th} measured value from the i^{th} method, m is the number of methods, n_i is the number of measurements from the i^{th} method, the symbol "~" signifies "is distributed as", and $N(0,\sigma^2)$ describes a normal (Gaussian) distribution with mean of zero and standard deviation of σ.

A Monte Carlo approach, consistent with the "Guide to the expression of uncertainty in measurement" (GUM) and its Supplement 1 [17,18], was used to compute the expanded uncertainty for each analyte. One million randomly generated sets of estimated method means were created and the DerSimonian-Laird estimated consensus value calculated for each set:

$$\boldsymbol{\bar{x}_{ij}} \;=\; \boldsymbol{\hat{\mu}_{\mathrm{DL}} + u.meth_i + u.meas_{ij}} \begin{cases} \boldsymbol{i = 1, \ldots, 10^6} \\ \boldsymbol{j = 1, \ldots, n} \end{cases} \tag{9}$$

where $\bar{x}_{ij}$ are the generated method means, $\hat{\mu}_{\mathrm{DL}}$ is the DerSimonian-Laird estimated consensus value based on the measurement results, $u.meth_i$ is the within method uncertainty component, $u.meth_i$ is the between-method uncertainty component, and n is the number of methods included in the analysis.

The within-method uncertainty component is estimated as:

$$\boldsymbol{u.meas_{ij}} = \frac{(\boldsymbol{m_j}-\mathbf{1})\boldsymbol{u_j^2}}{\boldsymbol{C_i}} \begin{cases} \boldsymbol{i} = \mathbf{1}, \ldots, \mathbf{10^6} \\ \boldsymbol{j} = \mathbf{1}, \ldots, \boldsymbol{n} \end{cases} \tag{10}$$

where (m_j - 1) is the degrees of freedom associated with the value and uncertainty provided by the j^{th} method, u_j is the standard uncertainty for the value provided by the j^{th} method, and C_i is a value drawn at random from a $\chi^2_{\boldsymbol{m_j-1}}$ distribution.

The between-method uncertainty component is defined:

$$\boldsymbol{u.meth_i} \sim \boldsymbol{N}(\mathbf{0}, \boldsymbol{\beta_i^2});\ \boldsymbol{\beta_i^2} \sim \boldsymbol{A_i}\left(\frac{\tilde{\boldsymbol{m}}\hat{\boldsymbol{\tau}}^{\mathbf{2}}_{\boldsymbol{DL}} + \hat{\boldsymbol{\sigma}}^{\mathbf{2}}}{\tilde{\boldsymbol{m}}(\boldsymbol{n}-\mathbf{1})}\right) - \boldsymbol{B_i}\left(\frac{\hat{\boldsymbol{\sigma}}^{\mathbf{2}}}{\tilde{\boldsymbol{m}}\boldsymbol{n}(\tilde{\boldsymbol{m}}-\mathbf{1})}\right) \tag{11}$$

where $\tilde{m}$ is one more than the median degrees of freedom across the n methods, $\hat{\tau}^2_{DL}$ is the DerSimonian-Laird estimated between-method variance, $\hat{\sigma}^2$ is the median estimate of the within method measurement variance, A_i is a value drawn at random from a χ^2_{n-1} distribution, and B_i is a value drawn at random from a $\chi^2_{n(\tilde{m}-1)}$ distribution [15, pg. 69].

For a given simulated set of method means, the $u.meth_i$ is constant across the (j = 1,…,n) methods while the $u.meth_{ij}$ varies by method.

From this empirical distribution of consensus values, a symmetrical 95 % coverage interval such that the upper and lower bounds are equidistant from the estimated consensus value based on the measured data was found. The half-width of this coverage interval provides the expanded uncertainty. The standard deviation of the empirical distribution provides the standard uncertainty.

4.3. Unit Conversions

NIST's measurements were made using gravimetric preparation and originally recorded as mass fractions (w) in units of mg/g. The clinical communities are more familiar with values expressed as mass concentrations (x) in units of g/dL and amount concentrations (c) in units of mmol/L.

Using the measured serum densities (ρ_{22}) listed in Table 6, NIST's w in units of mg/g were converted to mass concentrations (x) in units of mg/dL:

$$\left(\boldsymbol{x}\ \frac{\mathbf{mg}}{\mathbf{dL}}\right) = \left(\boldsymbol{w}\ \frac{\mathbf{mg}}{\mathbf{g}}\right)\left(\boldsymbol{\rho_{22}}\frac{\mathbf{g}}{\mathbf{mL}}\right)\left(\frac{\mathbf{100\ mL}}{\mathbf{dL}}\right). \tag{12}$$

The mass concentrations in units of mg/dL are related to amount concentrations (c) in units of mmol/L through the molar mass (atomic weight) of the analyte (M):

$$\begin{aligned} \left(\boldsymbol{c}\ \frac{\mathbf{mmol}}{\mathbf{L}}\right) &= \frac{\left(\boldsymbol{x}\frac{\mathbf{mg}}{\mathbf{dL}}\right)}{\left(\boldsymbol{M}\ \frac{\mathbf{g}}{\mathbf{mol}}\right)}\left(\frac{\mathbf{g}}{\mathbf{1000\ mg}}\frac{\mathbf{1000\ mmol}}{\mathbf{mol}}\frac{\mathbf{10\ dL}}{\mathbf{L}}\right) \\ \left(\boldsymbol{x}\ \frac{\mathbf{mg}}{\mathbf{dL}}\right) &= \left(\boldsymbol{c}\frac{\mathbf{mmol}}{\mathbf{L}}\right)\left(\boldsymbol{M}\ \frac{\mathbf{g}}{\mathbf{mol}}\right)\left(\frac{\mathbf{1000\ mg}}{\mathbf{g}}\frac{\mathbf{mol}}{\mathbf{1000\ mmol}}\frac{\mathbf{L}}{\mathbf{10\ dL}}\right). \end{aligned} \tag{13}$$

The currently accepted elemental molar masses are accessible at [19]; Table 7 lists the values and associated uncertainties for the seven analytes of interest in the SRM 956e materials.

Table 7. Molar Masses for the Seven Analytes of Interest.

		g/mol			
Element	Symbol	Molar Mass	*u*	*U*	U_{rel}, %
Lithium	Li	6.967	0.017	0.027	0.39
Sodium	Na	22.989 669 280	0.000 000 012	0.000 000 019	0.000 000 083
Magnesium	Mg	24.305 50	0.000 90	0.001 40	0.0058
Phosphorous	P	30.973 761 995 0	0.000 000 002 9	0.000 000 004 7	0.000 000 015
Chlorine	Cl	35.4515	0.0032	0.0052	0.015
Potassium	K	39.098 300	0.000 060	0.000 090	0.000 23
Calcium	Ca	40.0780	0.0023	0.0038	0.0095
Bromine	Br	79.9040	0.0017	0.0029	0.0036

5. Sodium (Na)

Inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry, both following a single-point standard additions protocol, were used to measure the sodium mass concentration of the SRM 956e materials. The two methods were used to evaluate different ampoules of the SRM 956e materials.

5.1. Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)

5.1.1. Materials

Twelve randomly selected ampoules of SRM 956e Level 1 and ten ampoules each of Levels 2 and 3 were analyzed. Two ampoules of each SRM 956d Level were analyzed as control materials. SRM 3124a Indium (In) Standard Solution [20] and SRM 3153a Strontium (Sr) Standard Solution [21] were used as internal standards (IS). SRM 3152a Sodium (Na) Standard Solution [22] was used as the standard addition spike. Optima grade nitric acid (HNO_3) was used for digestion. All water used was collected from an in-house 18 MΩ·cm water source.

5.1.2. Equipment

A Perkin-Elmer Optima 5300 Dual View inductively coupled plasma optical emission spectrometer (ICP-OES) was used for the analysis. A calibrated Mettler AT261 Delta Range analytical balance was used for weighing in the preparation of samples and standards; calibration was verified before initial use. Samples were digested using MARSXpress vessels in a Mars 5 Microwave Digestion system (CEM Corp., Matthews, NC USA).

5.1.3. Sample Preparation

For each Level, twelve (Level 1) or ten (Levels 2 and 3) of SRM 956e and two ampoules of SRM 956d were removed from -80 °C storage. Once vials were completely thawed after ≈2 h, two 1 g aliquots were taken from each ampoule, weighed and placed in Teflon microwave vessels. Eight procedural reagent blanks were prepared along with the samples. To each vessel, 10 mL of concentrated HNO_3 and 0.35 mL of 100 µg/g solution of In were added.

Samples were digested in two steps: 1) from ambient to 120 °C over 20 min at 800 W power then held for 20 min, 2) from 120 °C to 185 °C over 20 min at 1600 W power then held for 20 min. After microwave digestion, solutions were transferred to polyethylene bottles and diluted to 30 g using 18 MΩ·cm water.

Weighed 0.75 g aliquots were taken from each original solution and transferred to polyethylene bottles. One milliliter of a solution containing 90 µg/g Sr was added to each polyethylene bottle as the IS for Na. Each solution was diluted to a final mass of 30 g with 18 MΩ·cm water.

Samples were analyzed after each level was prepared.

5.1.4. Single-Point Standard Additions with Use of an Internal Standard

The Na mass fraction was quantified by the method of single-point standard addition with use of an internal standard. In this method, the analytical instrument is calibrated by measuring the increase in the analytical signal that occurs when a known amount of the analyte is added to the sample. It avoids multiplicative types of matrix interferences (enhancements or depressions) since the calibrant is present with the same matrix as the sample. Each prepared sample is split into two portions and analyzed separately. One of the portions is spiked with a known mass of the analyte of interest, the other portion is not spiked.

The mass fraction of the analyte, w_{sam}, is then estimated as:

$$\boldsymbol{x_{sam}} = \left(\frac{\boldsymbol{R_u}}{\boldsymbol{R_{sp} - R_u}}\right)\left(\frac{\boldsymbol{m_{sp}}}{\boldsymbol{m_{sam}}}\right)(\boldsymbol{w_{sp}})(\boldsymbol{F_{dil}}) \tag{14}$$

where R_{sp} and R_u are the internal-standard-corrected count rate of the spiked and the unspiked portions, m_{sam} and m_{sp} are the mass of the sample and the mass of the spike solution, w_{sp} is the mass fraction of the analyte in the spike solution, F_{dil} is the dilution factor of the sample.

The standard uncertainty and the approximate 95 % level of confidence expanded uncertainty of a series of measurements are calculated as:

$$\begin{aligned} \boldsymbol{u_{sam}} &= \sqrt{\boldsymbol{u_{reps}^2 + u_{blank}^2} + \left(\sum_{\boldsymbol{i}} \boldsymbol{u_i^2}\right)} \\ \boldsymbol{U_{sam}} &= \boldsymbol{k \times u_{sam}} \end{aligned} \tag{15}$$

where u_{reps} is the standard uncertainty of the mean of n_{reps} sample measurements, u_{blank} is the standard uncertainty of the mean of n_{blank} blank measurements, the u_i represent the standard uncertainties associated with other identified components of uncertainty, and k is the Student's t coverage factor for a 95 % confidence level for a v_{sam} degrees of freedom.

5.1.5. Sample Analysis

All samples were analyzed in as-received condition using the instrument's radial plasma view, integration time of 0.1 s, read time of 1 s, and wavelengths 589.592 nm for Na and 460.733 nm for Sr. Samples were diluted so that the analyte was present at an appropriate mass fraction. From each dilution, two aliquots were taken, with a Na spike prepared from SRM 3152a added to one. All samples were measured on day 1, and the measurement was repeated on the second day. The two-day average for each sample was reported after a one-way analysis of variance (ANOVA) showed no statistical difference in results from the two days. A one-way ANOVA of the results of duplicate samples from each vial at each Level showed no statistical difference between vials, indicating no detectable heterogeneity of Na in SRM 956e.

The results for the SRM 956d materials agreed with the certified values [5] with no statistically significant differences.

The ICP-OES results for Na in the SRM 956e materials are displayed and summarized in Table 8, with “Sample A” and “Sample B” denoting results from the two portions of the same samples. The results are displayed in Fig. 5 as functions of the ampoule index.

Table 8. ICP-OES Sodium Measurement Results for SRM 956e, mg/dL.

	Level 1		Level 2		Level 3	
	Sample A	Sample B	Sample A	Sample B	Sample A	Sample B
1	283.3	286.6	332.7	328.4	376.4	373.1
2	278.3	280.8	324.8	321.8	374.1	375.3
3	279.5	278.5	322.8	323.7	374.6	368.2
4	283.7	277.9	330.1	[a]	368.5	368.9
5	278.4	283.8	330.7	[a]	374.7	370.5
6	279.1	279.5	322.4	328.8	367.9	368.9
7	279.5	279.3	329.6	327.8	370.9	378.2
8	281.1	283.1	324.7	340.6	375.2	376.7
9	294.3	284.2	321.6	327.4	379.4	372.0
10	279.7	280.5	322.9	322.2	374.6	373.4
11	278.4	282.9				
12	283.0	280.2				
N:	24		18		20	
Mean:	281.5		326.8		373.0	
Standard Deviation:	3.6		4.9		3.5	
u_{reps} [b]:	0.73		1.16		0.74	
u_{blank} [b]:	0.36		0.27		0.33	
u_{spike} [b]:	0.27		0.31		0.36	
u_{weigh} [b]:	0.011		0.011		0.012	
u [b]:	0.86		1.23		0.89	
v [b]:	36		21		35	
k [b]:	2		2.08		2.00	
U [b]:	1.7		2.5		1.8	

a) Sample lost during preparation.
b) See Section 5.1.4 for the definition of symbols.

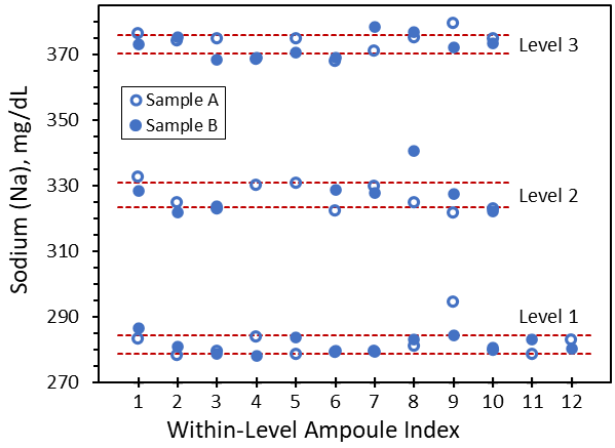

Fig. 5. ICP-OES Sodium (Na) Measurements for SRM 956e.

Solid circles represent the Sample A results; open circles represent Sample B results. Dashed horizontal lines bound the mean ± standard deviation intervals.

5.2. Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

5.2.1. Materials

Nine randomly selected ampoules of SRM 956e Level 1 and eight ampoules each of Levels 2 and 3 were analyzed. One ampoule of each SRM 956d Level was analyzed as control material. The ampoules were selected using stratified random sampling across the production sequence. SRM 3141a Potassium (K) Standard Solution [23] was used as an internal standard. SRM 3152a Sodium (Na) Standard Solution [22] was used as the standard additions spike. Optima grade nitric acid (HNO_3) was used for sample preparation. Locally prepared sub-boiling distilled water was used as the solvent for the preparation of all solutions.

5.2.2. Equipment

An Agilent model 7500cs inductively coupled plasma mass spectrometer (ICP-MS) was used for the analysis. A calibrated Mettler AT261 DeltaRange analytical balance was used for weighing in the preparation of samples and standards; calibration was verified before initial use.

5.2.3. Sample Preparation

One 0.3 g aliquot from each of nine ampoules of SRM 956e level 1 and four 0.3 g aliquots from one ampoule of SRM 956d level 1 were transferred into separate 60 mL low-density polyethylene (LDPE) bottles. One 0.25 g aliquot from each of eight ampoules of SRM 956e level 2 serum and four 0.25 g aliquots from one ampoule of SRM 956d level 2 serum were transferred into separate 60 mL LDPE bottles. One 0.22 g aliquot from each of 8 ampoules of SRM 956e level 3 serum and four 0.22 g aliquots from one ampoule of SRM 956d level 3 serum were transferred into separate 60 mL LDPE bottles. The content in each bottle was diluted to 50 g with 1.5 % volume fraction HNO_3. Four procedural blanks were prepared similarly, except that SRM 3141a K was added to the blanks to reach a mass fraction of 1.2 µg/g serving as an internal standard. No K was added to the samples as the native K in the serum served the purpose. A spiked sample was prepared for each ampoule and blank by transferring into a 30 mL LDPE bottle a 25 g aliquot from the unspiked sample and a 0.5 g aliquot of a solution containing 1 mg/g Na.

5.2.4. Sample Analysis

All samples were measured in the quantitative analysis mode using He as collision gas. Na was measured at 23 Da. K was measured at 39 Da. The analyte mass fraction was quantified by the method of single-point standard addition with use of an internal standard as outlined in Section 5.1.4.

The results for the SRM 956d materials agreed with the certified values [5] with no statistically significant differences.

The ICP-MS results for Na in the SRM 956e materials are displayed and summarized in Table 9. The results are displayed in Fig. 6 as functions of the ampoule index.

Table 9. ICP-MS Sodium (Na) Measurement Results for SRM 956e, mg/dL.

	Level 1	Level 2	Level 3
1	281.1	325.8	370.4
2	280.4	327.6	376.4
3	282.6	323.9	372.0
4	281.3	327.2	371.8
5	277.2	326.3	373.3
6	283.8	326.5	374.7
7	283.7	327.8	369.7
8	284.1	325.5	372.0
9	280.7		
N:	9	8	8
Mean:	281.7	326.3	372.5
Standard Deviation:	2.2	1.3	2.2
u_{reps} [a]:	0.73	0.45	0.78
u_{blank} [a]:	0.012	0.017	0.022
$u_{calibrant}$ [a]:	0.27	0.31	0.35
$u_{weighing}$ [a]:	0.045	0.062	0.081
$u_{density}$ [a]:	0.032	0.029	0.024
u [a]:	0.78	0.55	0.86
v [a]:	10	15	10
k [a]:	2.23	2.13	2.23
U [a]:	1.7	1.2	1.9

a) See Section 5.1.4 for the definition of symbols

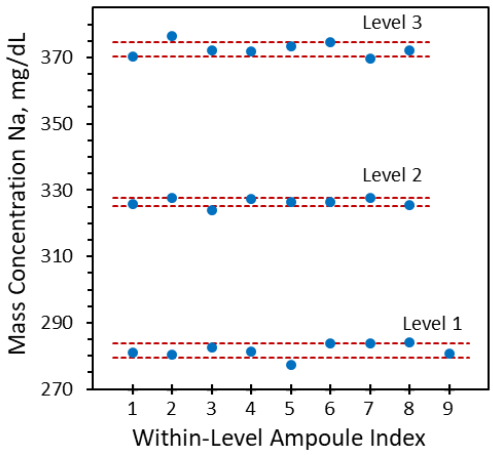

Fig. 6. ICP-MS Sodium (Na) Measurements for SRM 956e.

Each symbol denotes the result of one ICP-MS measurement. Dashed horizontal lines bound the mean ± standard deviation intervals.

5.3. Consensus

The consensus results for Na in SRM 956e Levels 1 to 3 using the multi-method analysis described in Section 4.2 are depicted in Fig. 7. Table 10 lists the mass concentration values; it also presents amount concentration values, derived using Eq. 13 and the Na molar mass listed in Table 7. These results are traceable to the SI through use of two independent reference measurement procedures and use of primary calibration standards derived from SRM 3100 series certified reference materials (CRMs) [7].

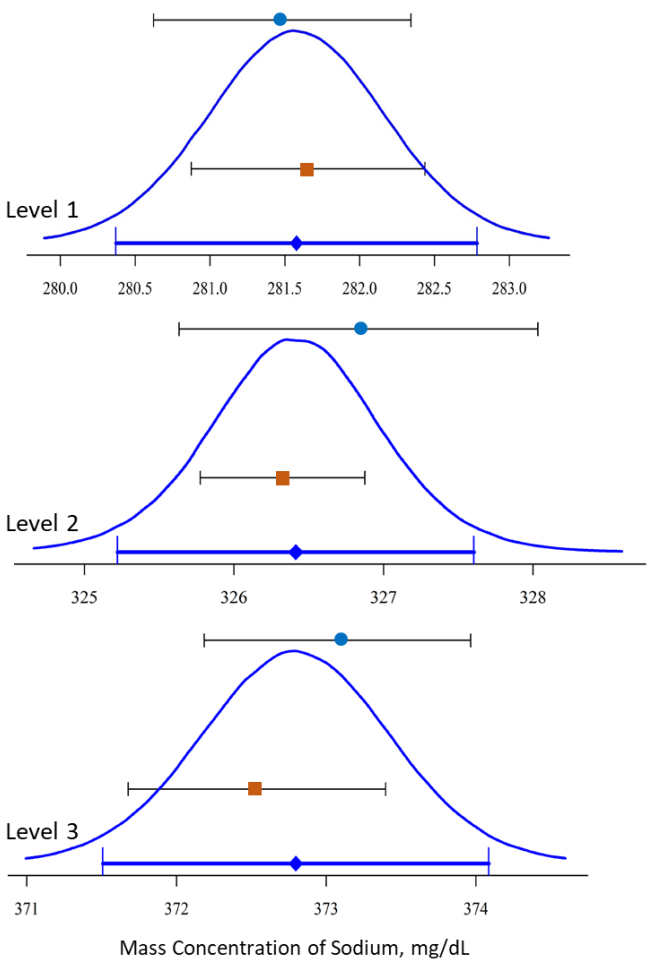

Fig. 7. Consensus Sodium Mass Concentration Results for SRM 956e.

Circles represent the ICP-OES mean results with their associated standard uncertainties. Squares represent ICP-MS mean results with their associated standard uncertainties. Curves represent the empirical distribution of consensus values; diamonds represent the final consensus values with their associated approximately 95 % level of confidence symmetric coverage intervals.

Table 10. Sodium Mass and Amount Concentrations of SRM 956e.

	mg/dL			mmol/L		
	x_{Na}	$u(x_{Na})$	$U(x_{Na})$	c_{Na}	$u(c_{Na})$	$U(c_{Na})$
Level 1	281.58	0.61	1.21	122.48	0.27	0.53
Level 2	326.41	0.60	1.19	141.98	0.26	0.52
Level 3	372.80	0.65	1.29	162.16	0.28	0.56

6. Potassium (K)

Potassium was evaluated using high-resolution isotope dilution sector field inductively coupled plasma mass spectrometry (ID-SF-ICP-MS). Because K suffers from ArH^+ isobaric interferences, the ID-ICP-MS method used for Li, Mg, and Ca in Section 7 cannot resolve the interference while the sector field technique can.

6.1. Materials

Eight randomly selected ampoules of each level of SRM 956e were selected for analysis. Two ampoules of each level of SRM 956d were used as control materials. A nominal 125 µg/g ^{41}K isotopic (99.18 ± 0.1) % enriched spike solution was purchased from Oak Ridge National Laboratory. Standard Reference Material 3141a Potassium Standard Solution [24] was used to calibrate the ^{41}K isotopic spike solution.

A makeup solution was prepared containing Fisher Optima grade ammonium hydroxide and Fisher Triton X-100 surfactant. The concentrated nitric acid (HNO_3) used was Fisher Optima grade. All water used was > 18 MΩ·cm water output from a Millipore Element DI water system with > 1 MΩ·cm feedwater input.

6.2. Sample Preparation

A complete description of the ID-ICP-MS sample preparation scheme utilized is described elsewhere [25]. The procedure involves spiking nominal 0.3 g test portions of the SRM materials with aliquots of the isotopic spike solution ranging from about (0.15 to 0.6) g for isotope dilution quantification. A calibrated five-place balance was utilized to measure the masses of sample and spike test portions by difference; the calibration was validated before beginning measurements.

The samples were initially diluted to a nominal volume of 50 mL, using a solution cocktail of 0.1 % ammonium hydroxide and 1 % Triton X-100 volume fraction in water to avoid sample coagulation. Subsequent volumetric dilutions were performed by subsampling 2 mL of the SRM Level 1 materials, 3 mL of the Level 2 materials, and 6 mL of the Level 3 materials and diluting to 50 mL using ≈ 2 % volume fraction of high-purity concentrated nitric acid in water. Varying levels of a makeup solution containing 1 % Triton X-100 were added to the Level 1 and 2 samples during dilution to ensure that each analytical sample contained nominally the same ≈ 0.12 % Triton-X100 in ≈ 1.76 % volume fraction nitric acid.

Similar procedures were followed to prepare six ID-MS procedural blanks and a matching background correction solution. Spike calibration samples and matching background correction samples were prepared only in the ≈ 2 % nitric acid solution to reduce any potential K contamination.

6.3. Instrumental Method

All prepared samples were measured in high resolution mode on an Element XR Inductively Coupled Plasma Mass Spectrometry (ICP-MS) system. All K isotope data were collected in the pulse counting regime using counting detector mode. A detector dead time of 27 ns was determined in advance of measurements and programmed into the instrument software to correct the raw sample count rates before exporting the data for offline calculations. The number of spectra per sample was set at 150. Except for mass range, the same instrument method parameters were used for ^{39}K and ^{41}K isotopes: Mass Window (100 %), Magnet Mass (38.963 Da), Settling Time (0.001 s), Segment Duration (0.500 s), Sample Time (0.010 s), Samples/Peak (40), Runs (3), Passes (50), Scan Type (E Scan), Search Window (60 %), Integration Window (10 %), Integration Type (Average). The mass range for ^{39}K was (38.961 to 38.966) Da, the range for ^{41}K was (40.959 to 40.964) Da.

6.4. Sample Analysis

SRM 3141a was used to calibrate the ^{41}K isotopic spike solution via reverse isotope dilution prior to use. Calibration and spike solutions were prepared in the 2 % nitric acid solution. Four gravimetric blends of the natural K (nominal 1 g aliquots of SRM 3141a) and enriched ^{41}K (nominal 0.44 g aliquots) solutions were produced and measured (target ^{41}K/^{39}K ratios near 1.3, corresponding to an approximate error magnification factor of 1.1). Spike calibration samples were run concurrently with the analytical samples. A gravimetrically prepared 13-fold dilution of the spike solution was used to spike the six procedural blanks.

6.4.1. Measurement Equation

The amount concentration of K in the analytical samples was calculated using the measurement function:

$$c_{\mathrm{x}} = \left(w_{\mathrm{z}} \frac{m_{\mathrm{y}}}{m_{\mathrm{x}}} \frac{m_{\mathrm{z}}}{m'_{y}} \left(\frac{K_{\mathrm{y1}} R_{\mathrm{y1}} - K_{\mathrm{b}} R_{\mathrm{b}}}{K_{\mathrm{b}} R_{\mathrm{b}} - K_{\mathrm{x1}} R_{\mathrm{x1}}} \right) \left(\frac{K'_{\mathrm{b}} R'_{\mathrm{b}} - K_{\mathrm{z1}} R_{\mathrm{z1}}}{K_{\mathrm{y1}} R_{\mathrm{y1}} - K'_{\mathrm{b}} R'_{\mathrm{b}}} \right) \left(\frac{K_{\mathrm{x1}} R_{\mathrm{x1}} + K_{\mathrm{x2}} R_{\mathrm{x2}}}{K_{\mathrm{z1}} R_{\mathrm{z1}} + K_{\mathrm{z2}} R_{\mathrm{z2}}} \right) - w_{\mathrm{B}} \right) \frac{\rho}{M} C_{\mathrm{b}} C_{\mathrm{r}} C_{\mathrm{t}} \tag{16}$$

where: c_x = amount concentration of K in samples (mmol/L),
C_b = 1, used to incorporate background subtraction uncertainty,
C_r = 1, used to incorporate replication uncertainty,
C_t = 1, used to incorporate background detector dead time uncertainty,
K_b = mass bias correction of R_b,
K_b' = mass bias correction of R_b',
K_{x1} = mass bias correction of R_{x1},
K_{x2} = mass bias correction of R_{x2},
K_{y1} = mass bias correction of R_{y1},
K_{z1} = mass bias correction of R_{x1},
K_{z2} = mass bias correction of R_{x2},

m_x = mass of sample in ID-MS blend solution (g),
m_y = mass of spike in ID-MS blend solution (g),
m_y' = mass of spike in spike calibration solution (g),
m_z = mass of SRM 3141a in spike calibration solution (g),
M = molar mass of K (g/mol),
R_b = measured $^{41}K/^{39}K$ ratio in ID-MS blend solution,
R_b' = measured $^{41}K/^{39}K$ ratio in spike calibration solution,
R_{x1} = measured ratio of enriched isotope to reference isotope ($^{41}K/^{39}K$) in sample,
R_{x2} = $^{40}K/^{39}K$ ratio in sample using literature value for ^{40}K [19],
R_{y1} = measured $^{41}K/^{39}K$ ratio in the ^{41}K enriched spike material,
R_{z1} = measured $^{41}K/^{39}K$ ratio in SRM 3141a,
R_{z2} = $^{40}K/^{39}K$ ratio in SRM 3141a using literature value for ^{40}K [19],
w_z = mass fraction K in SRM 3141a primary standard (μg/g),
w_B = mass fraction K in procedure blank (μg/g),
ρ = serum density (g/mL).

Equation 16 is an elaboration of the measurement function derived in [26, Example A7]. It has been modified to specify the K in SRM 3141a in its native units (mass fraction) and to include terms for instrument background correction, sample density, molar mass of K, and sample replication, and detector dead time correction.

All signals for unknowns, controls, and procedural blanks were corrected for instrument detector dead time, K background, and mass discrimination/bias to establish the K isotope ratios used in Eq. 16. Background correction consisted of running a background correction solution (without analyte) before and after the procedural blanks, and similarly, bracketing between each level series of SRM 956e samples and corresponding SRM 956d control samples. Third-order polynomial fits were generated to model the changes in the background signals of the ^{39}K and ^{41}K isotopes as a function of time (sample order) to subtract the apparent background signals on a sample-by-sample basis. The mean background signals in units of counts-per-second (cps) for five measurements were (85 209 ± 4522) cps for ^{39}K and (36 182 ± 3243) cps for ^{41}K. These signals represented approximately 6 % and 2 % of the measured sample intensities for ^{39}K and ^{41}K, respectively.

A theoretical/experimental mass bias correction factor of (1.154 ± 0.006) was determined for further correction of the dead time-corrected and background-corrected K isotope ratios. A solution of SRM 3141a was used to experimentally measure the natural $^{39}K/^{41}K$ ratio, and IUPAC isotopic abundance data [19] was used to calculate the theoretical $^{39}K/^{41}K$ ratio value: ^{39}K = (0.932 581 ± 0.000 044) g/g and ^{41}K = (0.067 302 ± 0.000 044) g/g.

The absolute K procedural blanks ranged from ≈ (0.002 to 0.007) μg, with a mean blank of (0.004 ± 0.002) μg for six blanks. The average blank value was subtracted from the absolute K sample masses in μg for all SRM samples tested. Blank corrections were minor, ranging from approximately 0.003 % relative for SRM 956e Level 1, to 0.01 % relative for SRM 956d Level 3. The blank standard deviation was converted to a mole fraction of 0.000 343 μmol/g.

6.4.2. Measurement Results

The ID-SF-ICP-MS results for K in the SRM 956e materials are displayed and summarized in Table 11. The results are displayed in Fig. 6 as functions of the ampoule index.

Table 11. Potassium (K) Amount Concentrations of SRM 956e, mmol/L.

	Level 1	Level 2	Level 3
1	5.956	3.928	1.990
2	5.986	3.976	1.997
3	5.948	3.980	2.001
4	5.979	3.969	1.988
5	5.995	3.987	1.993
6	5.982	3.968	2.004
7	6.002	3.963	1.998
8	5.978	3.980	2.013
Mean:	5.978	3.969	1.998
Standard Deviation:	0.018	0.018	0.008

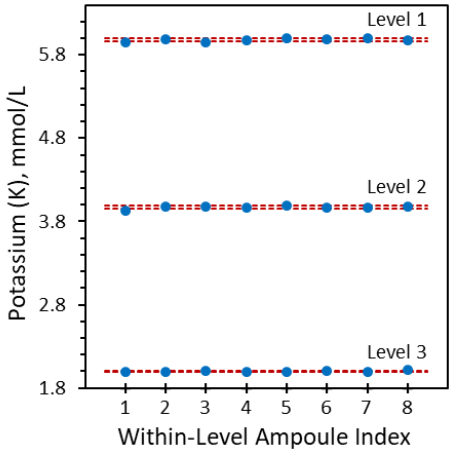

Fig. 8. ID-SF-ICP-MS Potassium (K) Measurements for SRM 956e.

Each symbol denotes the result of one ID-SF-ICP-MS measurement. Dashed horizontal lines bound the mean ± standard deviation intervals.

6.4.3. Uncertainty Estimation

A Kragten spreadsheet approach [12] was used to quantify combined standard uncertainties and to calculate expanded uncertainties using the measurement function for a typical sample, for each level of SRM 956e and SRM 956d. Table 12 lists the basis for assigning the standard uncertainties. The individual uncertainty components that were fed into the Kragten spreadsheets were determined according to ISO guidelines [17].

Table 12. Basis for the Values of the Measurement Function Standard Uncertainties.

Quantity	Uncertainty Description	Type	Distribution
$u(C_b)$	% RSD based on the quotients of standard deviations of ^{41}K and ^{39}K background signals measured at five time points, and the respective average ^{41}K and ^{39}K signals in the analytical samples, with individual % RSDs combined in quadrature	A	normal
$u(C_r)$	% RSD due to replication for *n* processed samples	A	normal
$u(C_t)$	% RSD of $^{41}K/^{39}K$ isotope ratio, based on the average measured experimental count rates for ^{41}K and ^{39}K. A 10K trial Monte Carlo simulation of detector dead time correction calculations, using a detector dead time setting of 27 nS ± 1 nS	B	uniform
$u(K_b)$	assume 0.5 % of quantity value (typical measurement precision)	B	uniform
$u(K'_b)$	assume 0.5 % of quantity value (typical measurement precision)	B	uniform
$u(K_{x1})$	negligible, assume 0.000 01 % of quantity value	B	uniform
$u(K_{x2})$	negligible, assume 0.000 01 % of quantity value	B	uniform
$u(K_{y1})$	negligible, assume 0.000 01 % of quantity value	B	uniform
$u(K_{z1})$	negligible, assume 0.000 01 % of quantity value	B	uniform
$u(K_{z2})$	negligible, assume 0.000 01 % of quantity value	B	uniform
$u(M)$	uncertainty in atomic mass of K [19]	B	uniform
$u(m_x)$	two times the five-place balance readability of 0.000 01 g	B	uniform
$u(m_y)$	two times the five-place balance readability of 0.000 01g	B	uniform
$u(m'_y)$	two times the five-place balance readability of 0.000 01 g	B	uniform
$u(m_z)$	two times the five-place balance readability of 0.000 01g	B	uniform
$u(R_b)$	% RSD of 150 replicate spectral scans of a typical sample	A	normal
$u(R'_b)$	% RSD of 4 spike calibration solutions each measured three times	A	normal
$u(R_{x1})$	% RSD based on the IUPAC amount fraction uncertainties for ^{41}K and ^{39}K [19]	B	uniform
$u(R_{x2})$	negligible, assume 0.000 01 % of quantity value	B	uniform
$u(R_{y1})$	1 % of quantity value, based on ^{41}K spike Certificate	B	uniform
$u(R_{z1})$	% RSD based on the IUPAC amount fraction uncertainties for ^{41}K and ^{39}K [19]	B	uniform
$u(R_{z2})$	negligible, assume 0.000 01 % of quantity value	B	uniform
$u(w_B)$	standard deviation of blank, $n = 6$	A	uniform
$u(w_z)$	SRM 3141a certified 95 % level of confidence expanded uncertainty	B	normal
$u(\rho)$	See Section 3.3	B	normal

6.5. Results

The results for all three levels of the SRM 956d control were in good agreement with the certified values.

Table 13 lists the K mass concentration and amount concentration values for the three levels of SRM 956e. These results are traceable to the SI through the ID-ICP-MS primary method, correction for all known bias sources, and use of primary standards derived from SRM 3100 series CRMs [7].

Table 13. Potassium Mass and Amount Concentrations of SRM 956e.

	mg/dL			mmol/L		
	x_K	$u(x_K)$	$U(x_K)$	c_K	$u(c_K)$	$U(c_K)$
Level 1	23.37	0.13	0.26	5.978	0.034	0.068
Level 2	15.52	0.09	0.18	3.969	0.023	0.046
Level 3	7.811	0.045	0.089	1.998	0.011	0.023

7. Lithium (Li), Magnesium (Mg), and Calcium (Ca)

Lithium (Li), magnesium (Mg), potassium (K), and calcium (Ca) were analyzed in the same prepared samples by isotope dilution inductively coupled plasma mass spectrometry (ID-ICP-MS). Because of ArH^+ isobaric interferences, the K results from this method are not used for value assigning K in SRM 956e materials (see Section 6.)

7.1. Materials

Eight randomly selected ampoules of SRM 956e of each Level were analyzed. Three ampoules of SRM 956d of each Level were analyzed as controls.

Two ampoules each of SRM 3109a Calcium Standard Solution [27], 3129a Lithium Standard Solution [28], SRM 3131a Magnesium Standard Solution [29], and SRM 3141a Potassium Standard Solution [24] were used to prepare two independent calibration solutions.

Working spike solution mixes containing ^{6}Li, ^{26}Mg, ^{41}K, and ^{42}Ca were prepared for each level from: nominal 200 µg/g 99.13 % ^{6}Li, nominal 420 µg/g 99.16 % ^{26}Mg, nominal 500 µg/g 99.18 % ^{41}K, and nominal 310 µg/g 94.41 % ^{42}Ca enriched isotope stock solutions purchased from Oak Ridge National Laboratory.

Reference Material 8545 LSVEC (Lithium Isotopes in Lithium Carbonate) [30] was used as a natural isotopic composition comparator for the $^{6}Li/^{7}Li$ ratio.

Optima grade nitric acid (HNO_3, Thermo Fisher) was used. High-purity water was prepared in-house by sub-boiling distillation using a conditioned, quartz still with deionized water as feedstock.

7.2. Equipment

A Thermo X series 2 ICP-MS system (Thermo Fisher Scientific, Waltham, MA) fitted with a 0.1 ml/min PFA ST microconcentric nebulizer (Elemental Scientific, Omaha, NE) was used for Li analysis. A Thermo X series 7 ICP-MS system (Thermo Fisher Scientific, Waltham, MA) fitted with a 1 ml/min glass concentric C-type nebulizer (Analytical West, Corona, CA) was used for Mg, K, and Ca analysis. Both systems were equipped with a Peltier cooled (2 °C) impact bead spray chamber and an ESI autosampler (Elemental Scientific, Omaha, NE).

All masses were obtained on a calibrated 5-place Mettler XP205 balance or calibrated 6-place Mettler XPE56 balance and recorded electronically. The balance calibrations were verified before initial use.

Samples were digested using TFM Teflon MARSEasyPrep digestion vessels with CEM Mars Microwave Digestion systems (CEM Corp., Matthews, NC USA).

All solutions were contained in LDPE bottles (Nalgene, Rochester, NY). Liquids were transferred using Reference® 2 (10 to 100) µL and (100 to 1000) µL adjustable pipettes with associated trace element grade tips (Eppendorf, Hamburg, Germany).

7.3. Sample Preparation

SRM ampoules contained approximately 2 g of serum. Levels were processed and analyzed in the following order: Level 3, Level 2, Level 1. For Level 3, single subsamples from each ampoule of SRM 956d and ampoules 1 to 6 of SRM 956e and two subsamples from ampoules 7 and 8 of SRM 956e were processed for isotope dilution analysis. Level 2, single subsamples from each ampoule of SRM 956d and ampoules 1 to 7 and two subsamples from ampoule 8 were processed. For Level 1, single subsamples from each ampoule of SRM 956d and ampoules 1 to 7 were processed.

Two composition samples, which did not undergo isotope spike addition, were taken from ampoules 1 and 2 of each Level of SRMs 956d and 956e.

Standard solutions composed of a 2 % volume fraction HNO_3 solution, containing known amounts of Li, Mg, K, and Ca at levels similar to each serum level, were processed in the same manner as the isotope dilution samples as a validation check on the accuracy of the measurement process. These are referred to as “standard as sample” (SAS) controls. Three SAS controls were processed for Level 3 and four SAS controls were processed for each of Levels 1 and 2. Four process blanks were included for each level.

Four spike calibration samples were prepared from two separate standard solution preparations of each element. These were used to calibrate the amount content of enriched ^{6}Li, ^{26}Mg, ^{41}K, and ^{42}Ca in the working spike solution mixes which were used for the isotope spike additions and process blanks.

7.3.1. Working Spike Solutions

At least 25 g of working spike solution mix was prepared for each level so that the elemental mass fractions of the spike solution mix matched the expected elemental mass fractions of SRM 956e samples. Aliquots of each enriched isotope stock solution were gravimetrically weighed into clean, tared LDPE bottles and gravimetrically diluted to volume with 2 % volume fraction HNO_3. Working spike solution mixes for Level 1 contained 14 µg/g ^{6}Li, 34 µg/g ^{26}Mg, 225 µg/g ^{41}K, and 125 µg/g ^{42}Ca. Working spike solution mixes for Level 2 contained 8 µg/g ^{6}Li, 23 µg/g ^{26}Mg, 150 µg/g ^{41}K, and 106 µg/g ^{42}Ca. Working spike solution mixes for Level 3 contained 4 µg/g ^{6}Li, 12 µg/g ^{26}Mg, 72 µg/g ^{41}K, and 83 µg/g ^{42}Ca.

The isotopic composition of each analyte in the working spike mix solutions was verified to be unchanged relative to the isotopic composition of the corresponding stock solution by ICP-MS analysis. In the case of Li, though the isotopic composition of the working spike mix and stock solution agreed, the measured isotopic compositions differed slightly from Oak Ridge assay and so the measured value was used in computations.

7.3.2. Spike Calibration Samples

Spike calibration samples were prepared concurrent with the analytical samples to have isotope ratios similar to the analytical samples. Known amounts of the working spike solution mix were added by mass difference via a capped plastic syringe to clean LDPE bottles. In a similar manner, two aliquots from each of two separate standard solution preparations were

gravimetrically added to the spike mix solution aliquots, resulting in four calibration samples per level for each element. For Mg, K, and Ca the standard solutions were prepared as a three-element mix by gravimetrically diluting appropriate amounts of the SRM 3100 series standards. Li standard solutions were prepared separately and added to four separate aliquots of working spike solution mix. The spike mixes and calibration standards were carefully mixed to equilibrate the isotopes by swirling and inverting the bottles.

7.3.3. Serum Samples

SRM 956d and 956e ampoules were removed from -80 °C storage and allowed to equilibrate to (19 to 21) ℃ ambient temperature for at least 120 min before sampling. The contents of each ampoule were mixed by gently inverting and rotating several times.

Test portion masses were obtained by difference. The neck of the ampoule was scored with a glass etcher, wiped with a damp cleanroom towel and snapped off. The contents of the ampoule were immediately withdrawn into a plastic syringe with an extended neck created by attaching a clean, nominal 4 cm long tip, which had been cut from a plastic disposable pipet. The syringe was weighed, nominally 0.5 g to 1.1 g was transferred to a clean microwave digestion vessel, and the syringe was reweighed.

7.3.4. Isotope Dilution Samples

An appropriate amount of the working spike solution mix was added gravimetrically to each test portion in the vessel by mass difference via a capped plastic syringe. Samples were spiked so that 1 µg of element in the spike mix was added for each 1 µg of element in the sample, resulting in isotope ratios near one.

7.3.5. Sample as Standard (SAS) Controls

The working spike solution mix was added to clean microwave digestion vessels. This was followed by the gravimetric addition of standard solutions so that again, the resulting isotope ratios were near one. Additional aliquots of the working spike solution mix were added individually by mass difference to clean LDPE bottles for purposes of preparing spike calibration samples. A smaller amount of the same spike mix solution that had been added to the serum and SAS samples was added to clean microwave digestion vessels to serve as procedure blanks.

7.3.6. Isotopic Standards

A dilute solution of the respective SRM 3100 standard solution was used as the isotopic standard for Mg, K, and Ca, and isotopic compositions reported in [31] were used. For Li, the natural isotopic composition is known to vary. As such the isotopic standard LSVEC (RM 8545), with assigned $^{6}Li/^{7}Li$ 95 % confidence interval of (0.082 15 ± 0.000 23) [30], was used.

7.4. Sample Digestion and Dilution

For each level, samples were processed in two sets of 12 vessels. Each set contained one SRM 956d composition sample, one SRM 956e composition sample, one to two SRM 956d isotope

dilution samples, five to six SRM 956e isotope dilution samples, two SAS isotope dilution samples, and two process blanks.

Ten grams of concentrated HNO_3 were added to each digestion vessel and samples were allowed to digest at ambient temperature for two hours prior to transfer to a CEM MARS microwave oven. The vessels were held at 830 kPa (120 psi) and heated in three stages: 1) from ambient to 120 °C over 20 min at 1600 W power then held for 20 min, 2) from 120 °C to ambient at 0 W power and held for 10 min (Levels 2 and 3) or 1.5 min (Level 1), 3) from ambient to 200 °C over 20 min at 1600 W power and held for 25 min.

After they had cooled, the vessels were removed from the microwave oven and their contents were transferred to clean 30 mL LDPE bottles with three vessel rinsings of 2 % volume fraction HNO_3 to a nominal dilution volume of 15 g. Samples were diluted further with 2 % volume fraction HNO_3 to reach the analysis mass fraction for each analyte. Li was measured separately in solutions diluted with 2 % volume fraction HNO_3 to contain (31 to 36) ng/g of Li. Mg was measured separately in solutions diluted with 2 % volume fraction HNO_3 to contain (17 to 25) ng/g of Mg. Ca and K were analyzed together with samples diluted to contain (220 to 300) ng/g Ca with K ranging from (220 to 450) ng/g.

Spike calibration samples were diluted to contain the same Li, Mg, K, and Ca mass fractions as the samples.

Blank samples were diluted by taking a similar sized aliquot as the samples and diluting to half the final volume of the samples with 2 % volume fraction HNO_3.

7.5. Inductively Coupled Plasma Mass Spectrometry.

ICP-MS analyses of Li in all isotope dilution samples (serum samples, controls, and spike calibration samples) were performed using a Thermo Elemental X2 system. ICP-MS analyses of Mg, K, and Ca were performed using a Thermo Elemental X7 system. Operating conditions were optimized daily based on the element or element pair being determined. All analyses were in Standard Mode, used Xt (matrix tolerant) cones, and 1400 W forward power. Other operating conditions are listed in Table 14.

Table 14. ICP-MS Operating Conditions for SRM 956e.

		Li, X2 System			Mg, X7 System			K and Ca, X7 System		
	Parameter	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3
	Peri-pump (rpm) blue/orange tubing	40	40	40	28	18	18	28	18	18
	H_2/He collision gas (mL/min)	0	0	0	0	0	0	8.5	8.5	8.5
Ar	Coolant (L/min)	13	13	13	13	13	13	13	13	13
Ar	Auxiliary (L/min)	0.9	0.9	0.9	0.9	0.9	0.9	0.9	1	1
Ar	Nebulizer (L/min)	0.92	0.93	0.93	0.86	0.87	0.85	0.86	0.9	0.9
Lens	Extraction (V)	-86.3	-78.4	-82.4	-235	-129	-98	-106	-114	-114
Lens	Lens 1 (V)	-1176	-941	-941	-0.6	-0.6	-0.6	-2.4	-2.4	-1.5
Lens	Focus/Collector (V)	11.8	14.3	14.3	16.7	16.7	12.4	6.7	6.7	6.7
Lens	D1 (V)	-44.7	-38.4	-39.2	-43.1	-47.1	-22.7	-39.2	-58	-58
Lens	Quadrupole Bias (V)	-4	-4	-4	0	0	-1	-6.3	-10	-10
Lens	Hexapole Bias (V)	-5	-3	-3	-5	-5	-6	-10	-9	-9
Lens	Lens 2 (V)	-83.1	-63.5	-65.9	-22.7	-22.7	-11	-57.3	-49.4	-49.4
Lens	Lens 3 (V)	-181.2	-181.2	-178.8	-160.8	-160.8	-140	-140.4	-140.4	-140.4
Lens	D2 (V)	-154	-143	-133	-160	-160	-91	-140	-140	-140
Lens	DA (V)	-34.5	-54.9	-54.9	-74.5	-74.5	-26.7	-74.5	-74.5	-74.5
Lens	Resolution (V)	100	110	110	115	115	105	85	95	95
Torch	Vertical	124	124	213	115	115	106	115	115	115
Torch	Horizontal	115	115	106	124	124	213	106	106	106
Torch	Sampling Depth	150	150	150	150	150	150	150	150	150

Measurements were made using peak jump data acquisition with one point per peak. Five blocks of data, each one minute in duration, were acquired per sample, and the mean ratios were used for computations. Measured ratios were corrected for mass bias, drift, and detector dead time.

A solution of 2 % volume fraction HNO_3 was measured at the start of an ICP-MS analysis sequence to correct all subsequent samples for instrument background. Following in the run sequence were dilutions of the stock enriched isotope solution, the spike mix solution, and the process blanks.

After an additional run of 2 % volume fraction HNO_3 to check for adequate analyte washout, an isotopic standard (a pure solution of natural isotopic composition) was measured. It was used to determine the mass bias correction applied to composition samples, enriched isotope solutions, and process blanks. After this, the measured mass bias correction factor was used to correct the measured ratio of one spike calibration sample.

The spike calibration sample, which had an isotopic ratio similar to the isotope dilution samples, was re-measured throughout the sequence of isotope dilution samples and was used to correct the remaining samples for mass bias and any subsequent instrument drift. Spike calibration samples were run at the beginning and end of the sequence of isotope dilution samples. Drift throughout the sequence was assessed every three samples and a correction applied by assuming the drift to be linear with time.

7.5.1. Measurement Function

The amount concentrations of Li, Mg, K, and Ca in the samples were calculated using an ID-MS measurement function derived from the equations in [32]:

$$\boldsymbol{c_{\mathrm{x}}} = \left(\frac{\boldsymbol{w_{\mathrm{y}} m_{\mathrm{y}}}}{\boldsymbol{m_x}} \left(\frac{\left(\boldsymbol{A_{\mathrm{y}}}\right)_{\mathrm{y}} - \left(\boldsymbol{A_{\mathrm{x}}}\right)_{\mathrm{y}} \times \boldsymbol{K_{\mathrm{b}}} \times \left(\boldsymbol{R_{\mathrm{y/x}}}\right)_{\mathrm{b}}}{\left(\boldsymbol{A_{\mathrm{x}}}\right)_{\mathrm{x}} \times \boldsymbol{K_{\mathrm{b}}} \times \left(\boldsymbol{R_{\mathrm{y/x}}}\right)_{\mathrm{b}} - \left(\boldsymbol{A_{\mathrm{y}}}\right)_{\mathrm{x}}} \right) - \boldsymbol{w_{\mathrm{blk}}} \right) \frac{\boldsymbol{\rho}}{\boldsymbol{M}} \boldsymbol{C_{\mathrm{rep}}} \qquad (17)$$

where: c_x = amount concentration of Li, Mg, K, and Ca in the samples (mmol/L):

A = isotope abundance,
b = sample blend (sample spiked with enriched isotopes),
blk = procedure blank,
C_{rep} = sample-to-sample repeatability,
K_b = correction factor for mass bias,
m = mass (g),
M = molar mass (g/mol),
R = ratio of spike isotope to reference isotope,
w = mass fraction (µg/g),
x = sample or reference (sample) isotope,
y = spike or spike isotope,
ρ = serum density (g/mL).

Table 15 lists the basis for assigning the standard uncertainties. The individual uncertainty components were determined according to ISO guidelines [17].

Table 15. Basis for the Values of the Measurement Function Standard Uncertainties.

Quantity	Uncertainty Description	Type
$u(C_{rep})$	Serum sample Repeatability	A
$u(w_{blk})$	Procedure blank repeatability	A
$u(R_{y/x})_b$	Uncertainty of intensity ratio at spike mass to reference mass in sample spiked with enriched isotope, combining ± 0.2 % dead time correction and an estimated mass spectrometric ratio bias based on the larger of the mean difference for the SAS or SRM 956d control sample runs relative to known values	B
$u(m_x)$	Assume ± 0.000 30 g in the measured sample mass	B
$u(m_y)$	Assume ± 0.000 30 g in the measured spike solution mass	B
$u(w_y)$	Uncertainty of the spike solution mass fraction calibrated by reverse ID, combining uncertainty of the primary standard (typically relative *U* between 0.17 % and 0.30 %), the difference between the results of the two primary standard solutions, and the standard error of the mean of the spike calibration samples.	B
$u((A_y)_y)$	Uncertainty of the abundance of spike isotope in the spike solution. The Oakridge certificate value used for Mg, K, and Ca. The Li value estimated from the measured ^{6}Li /^{7}Li of the spike solution.	B
$u((A_x)_y)$	Uncertainty of the abundance of reference isotope in the spike solution. The Oakridge certificate value used for Mg, K, and Ca. The Li value estimated from the measured ^{6}Li/^{7}Li of the spike solution.	B
$u((A_x)_x$	Uncertainty of the abundance of reference isotope in the sample. Literature values [31] were used for K, Ca and Mg. The Li value estimated from the measured ^{6}Li/^{7}Li used to calculate the ^{7}Li amount concentration of the sample.	B
$u((A_y)_x)$	Uncertainty of the abundance of reference isotope in the sample. Literature values [31] were used for Mg, K, and Ca. The Li value estimated from the measured ^{6}Li /^{7}Li used to calculate the ^{6}Li amount concentration of the sample.	B
$u(k_b)$	Uncertainty of the mass bias correction factor. Literature values [31] were used for K, Ca and Mg. The Li value estimated by combining the composition ratio measurement a relative ± 0.2 % based on experience.	B
$u(M)$	Uncertainty in natural elemental molar mass [19]	
$u(\rho)$	Uncertainty in serum density, see Section 3.3	B

7.5.2. Lithium (Li)

Lithium was analyzed in standard mode without a collision gas. Li signal intensities at ^{6}Li (spike isotope) and ^{7}Li (reference isotope) were measured at dwell times of 10 ms each. Quantitation was based on the ^{6}Li/^{7}Li ratio.

The isotopic composition of Li is known to vary due to geologic and anthropogenic processes. For this reason, it was necessary to measure the isotopic composition of the calibration materials and SRM 956d and SRM 956e serum samples. In addition, the isotopic composition of enriched ^{6}Li was measured. Atomic weights were calculated from the measured isotope abundances and using the nuclidic masses given in [33]. Standard uncertainties of the ^{6}Li/^{7}Li ratios were obtained from at least $n = 3$ separated analyses of each material.

The Li results are displayed and summarized in Table 16. The results are displayed in Fig. 9 as functions of the ampoule index.

Table 16. Lithium (Li) Measurement Results for SRM 956e (mmol/L).

	Level 1	Level 2	Level 3
1	2.0793	1.3019	0.5295
2	2.0818	1.3022	0.5300
3	2.0816	1.3048	0.5314
4	2.0783	1.3028	0.5293
5	2.0804	1.3015	0.5303
6	2.0792	1.3042	0.5257
7a	2.0803	1.3058	0.5309
7b			0.5336
8a		1.3025	0.5279
8b		1.3020	0.5293
N:	7	9	10
Mean:	2.0801	1.3031	0.5298
Standard Deviation:	0.0013	0.0015	0.0021
u(Type A):	0.000 49	0.000 50	0.000 66
u(Type B):	0.0086	0.0135	0.0067
u:	0.0086	0.0135	0.0067
v:	> 60	> 60	> 60
k:	2	2	2
U:	0.017	0.027	0.013

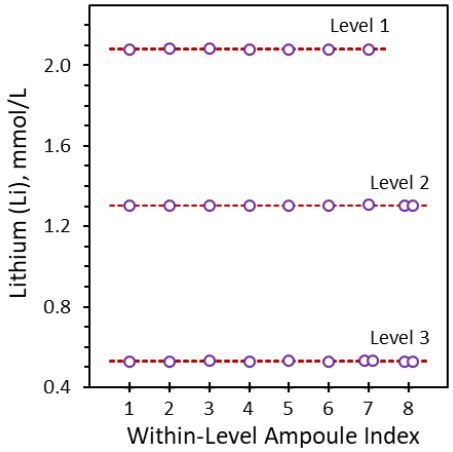

Fig. 9. Lithium (Li) Measurements for SRM 956e.

Each symbol denotes the result of one ID-ICP-MS measurement. Dashed horizontal lines bound the mean ± standard deviation intervals.

7.5.3. Magnesium (Mg)

Magnesium was analyzed in standard mode without a collision gas. Mg signal intensities at ^{24}Mg (reference isotope), ^{25}Mg, and ^{26}Mg (spike isotope) were measured at dwell times of 10 ms each. Quantitation was based on the ^{26}Mg/^{24}Mg ratio.

The Mg results are displayed and summarized in Table 17. The results are displayed in Fig. 10 as functions of the ampoule index.

Table 17. Magnesium (Mg) Measurement Results for SRM 956e, (mmol/L).

	Level 1	Level 2	Level 3
1	1.4621	0.9934	0.5285
2	1.4647	0.9912	0.5254
3	1.4637	0.9879	0.5288
4	1.4633	0.9904	0.5290
5	1.4608	0.9916	0.5263
6	1.4614	0.9899	0.5231
7a	1.4625	0.9895	0.5277
7b			0.5290
8a		0.9901	0.5262
8b		0.9901	0.5254
N:	7	9	10
Mean:	1.4626	0.9905	0.5270
Standard Deviation:	0.0014	0.0015	0.0020
u(Type A):	0.000 53	0.0017	0.000 63
u(Type B):	0.010	0.0045	0.0038
u:	0.010	0.0049	0.0038
v:	> 60	> 60	> 60
k:	2	2	2
U:	0.020	0.0097	0.0076

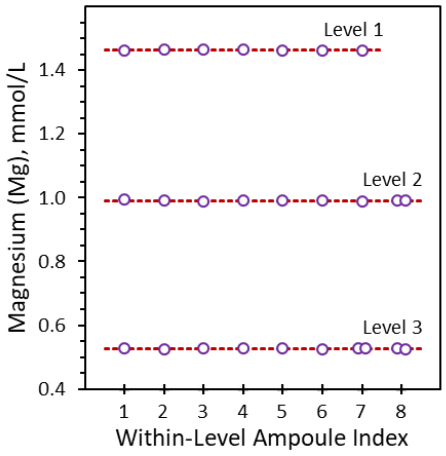

Fig. 10. Magnesium (Mg) Measurements for SRM 956e.

Each symbol denotes the result of one ID-ICP-MS measurement. Dashed horizontal lines bound the mean ± standard deviation intervals.

7.5.4. Calcium (Ca)

Ca was analyzed in collision cell mode using 8 % volume fraction H_2 in He as the collision gas. A small kinetic energy barrier, calculated as the difference between the quadrupole bias and hexapole bias voltages, was used. Calcium signal intensities were measured at ^{40}Ca (reference isotope) and ^{42}Ca (spike isotope) at dwell times of 10 ms each. Quantitation was based on the $^{42}Ca/^{40}Ca$ ratio. The signal intensity measured for ^{40}Ca was corrected for signal contribution from ^{40}K by multiplying the signal intensity measured at ^{39}K by the natural $^{40}K/^{39}K$ ratio measured in a standard solution of K. On average, the correction for ^{40}K was less than 0.4 % of the ^{40}Ca signal.

The ICP-MS results for Ca are displayed and summarized in Table 18. The results are displayed in Fig. 11 as functions of the ampoule index.

Table 18. Calcium (Ca) Measurement Results for SRM 956e, (mmol/L).

	Level 1	Level 2	Level 3
1	3.0308	2.5284	2.0220
2	3.0360	2.5106	2.0090
3	3.0311	2.5081	2.0340
4	3.0442	2.5177	2.0260
5	3.0192	2.5132	2.0260
6	3.0247	2.5132	2.0550
7a	3.0241	2.5192	2.0100
7b			2.0270
8a		2.5147	2.0270
8b		2.5064	2.0270
N:	7	9	10
Mean:	3.0300	2.5146	2.026
Standard Deviation:	0.0083	0.0066	0.013
u(Type A):	0.0038	0.0063	0.0051
u(Type B):	0.012	0.014	0.0070
u:	0.012	0.016	0.0087
v:	> 60	> 60	> 60
k:	2	2	2
U:	0.024	0.031	0.017

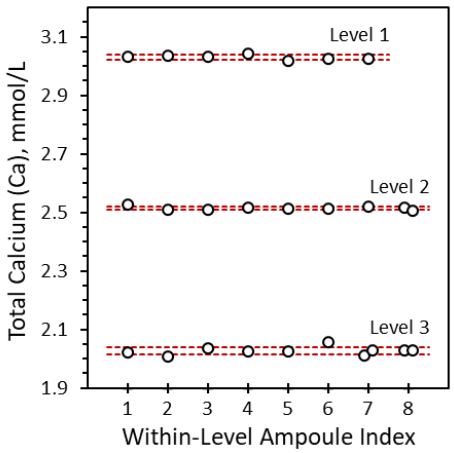

Fig. 11. Calcium (Ca) Measurements for SRM 956e.

Each symbol denotes the result of one ID-ICP-MS measurement. Dashed horizontal lines bound the mean ± standard deviation intervals.

7.6. Results

Results for Li, Mg, and Ca show that all elements appear to be distributed homogenously in SRM 956e Levels 1 to 3. The relative standard deviation between ampoules was no more than 0.64 %, which is about the expected mass spectrometric measurement repeatability.

The relative standard deviation for four spike calibration mixes (two each of two different primary standard solutions) is no more than 0.12 % for ^{6}Li, 0.24 % for ^{26}Mg, and 0.25 % for ^{42}Ca. The variability observed between the mixes is one measure of the uncertainty associated with gravimetric sample preparation and ICP-MS ratio measurement of a matrix-free sample.

The blank mean of results for each level was used to correct samples pertaining to that level. In some cases, measured Li process blanks were at the detection limit of the method and "negative" blanks were observed. In each case, the blank correction was computed as the mean of all positive blanks whereas the standard error of the mean was computed using all positive and negative blanks. Because the four Li blanks for Level 2 could not be distinguished from zero, a correction of (0 ± 0.037) ng (the standard error) was used. In all cases, the corrections for process blank were less than 1 %.

The results for Li, Mg, and Ca in the SRM 956d controls overlapped with their certified values.

Results for SAS samples are not affected by matrix-induced spectral interference. The SAS samples are prepared gravimetrically to contain Li, Mg, and Ca with an estimated relative expanded uncertainty of 0.3 % and are subject to all sample preparation and ICP-MS measurement steps. The overall mean % differences and % relative standard deviation from the gravimetric values were: (0.24 ± 0.70) % for Li, (-0.53 ± 0.72) % for Mg, and (-0.43 ± 0.73) % for Ca. While the results for Mg and Ca were not within the 0.3 % expanded uncertainty of the SAS preparation, the isotope dilution results reported here are accurate to an overall level of better than 1 % in the absence of spectral interference. The results for Li measured on the X Series 2 ICP-MS system show smaller differences for the SAS samples than the results for Mg and Ca

measured on the X Series 7 ICP-MS system which required repairs that rendered the instrument unavailable for much of the time in which these analyses were conducted.

Measured serum ratios for Li, Mg, and Ca agreed to within 1 % of the ratios measured for the isotopic standard of each element, indicating an absence of interferences in the mass regions of interest.

Table 19, Table 20, and Table 21 list the Li, Mg, and Ca mass concentration and amount concentration values for the three levels of SRM 956e. These are traceable to the SI by the use of an ID-ICP-MS primary method, correction for all known bias sources, and use of primary standards derived from SRM 3100 series CRMs [7].

Table 19. Lithium (Li) Mass and Amount Concentrations of SRM 956e.

	mg/dL			mmol/L		
	x_{Li}	$u(x_{Li})$	$U(x_{Li})$	c_{Li}	$u(c_{Li})$	$U(c_{Li})$
Level 1	1.4440	0.0060	0.012	2.0801	0.0086	0.017
Level 2	0.9045	0.0094	0.019	1.3031	0.0135	0.027
Level 3	0.3678	0.0047	0.0093	0.5298	0.0067	0.013

Table 20. Magnesium (Mg) Mass and Amount Concentrations of SRM 956e.

	mg/dL			mmol/L		
	x_{Mg}	$u(x_{Mg})$	$U(x_{Mg})$	c_{Mg}	$u(c_{Mg})$	$U(c_{Mg})$
Level 1	3.5550	0.025	0.049	1.4626	0.010	0.020
Level 2	2.4073	0.012	0.024	0.9905	0.049	0.0097
Level 3	1.2808	0.0092	0.018	0.5270	0.038	0.0076

Table 21. Calcium (Ca) Mass and Amount Concentrations of SRM 956e.

	mg/dL			mmol/L		
	x_{Ca}	$u(x_{Ca})$	$U(x_{Ca})$	c_{Ca}	$u(c_{Ca})$	$U(c_{Ca})$
Level 1	12.144	0.050	0.098	3.0300	0.012	0.024
Level 2	10.078	0.063	0.12	2.5146	0.016	0.031
Level 3	8.121	0.035	0.070	2.0263	0.0087	0.017

8. Phosphorous (P)

Inductively coupled plasma optical emission spectrometry (ICP-OES) following a single-point standard additions protocol was used to measure the phosphorous (P) mass concentration of the SRM 956e materials. The sample solutions analyzed were those prepared for the ICP-OES analysis of Na (Section 5).

8.1. Materials

The materials are described in Section 5.1.1 with the exception that SRM 3139a Phosphorous (P) Standard Solution [34] was used as the standard additions spike.

8.2. Equipment

A Perkin-Elmer Optima 8300 Dual View inductively coupled plasma optical emission spectrometer (ICP-OES) was used for the analysis. Otherwise, the equipment was as described in Section 5.1.2.

8.3. Sample Preparation

The digested sample solutions described in Section 5.1.3 before the addition of Sr were analyzed for P.

8.4. Sample Analysis

All samples were analyzed using the instrument's axial plasma view, integration time of 0.1 s, read time of 1 s, and wavelengths 213.617 nm for P and 230.606 nm for In. Otherwise, samples were analyzed and quantified as described in Section 5.1.5.

The ICP-OES results for P in the SRM 956e materials are displayed and summarized in Table 22, with "Day 1" and "Day 2" denoting results from two runs on the same samples on subsequent days. The results are displayed in Fig. 5 as functions of the ampoule index.

Table 22. Phosphorus (P) Measurement Results for SRM 956e, mg/dL.

	Level 1		Level 2		Level 3	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
1	13.34	13.52	10.98	11.70	9.78	8.71
2	13.31	13.77	11.63	11.46	10.34	8.34
3	13.49	12.99	11.63	11.87	11.61	10.98
4	13.86	12.92	11.73	*a*	9.45	8.55
5	13.22	13.07	11.06	*a*	8.63	8.28
6	13.39	13.02	11.04	10.49	8.21	8.55
7	12.97	12.77	11.18	11.22	7.75	9.51
8	12.69	13.39	11.53	12.09	9.67	9.79
9	14.03	12.15	11.23	11.09	9.82	11.30
10	13.10	12.58	11.01	11.48	11.61	9.77
11	12.77	13.78				
12	12.94	13.16				
N:	24		18		20	
Mean:	13.18		11.36		9.53	
Standard Deviation:	0.44		0.39		1.17	
u_{reps} [b]:	0.090		0.092		0.262	
u_{blank} [b]:	0.062		0.056		0.26	
u_{spike} [b]:	0.018		0.015		0.013	
$u_{weigh\ standards}$ [b]:	0.000 18		0.000 26		0.000 28	
$u_{weigh\ samples}$ [b]:	0.000 077		0.000 066		0.000 056	
u [b]:	0.111		0.109		0.369	
v [b]:	30		24		20	
k [b]:	2.04		2.06		2.09	
U [b]:	0.226		0.225		0.770	

a) Sample lost during preparation.
b) See Section 5.1.4 for the definition of symbols.

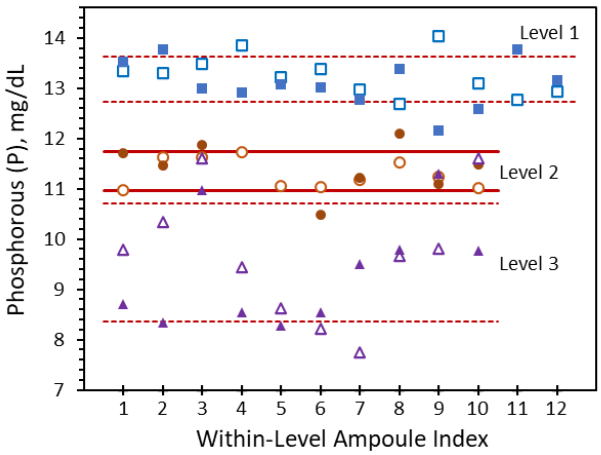

Fig. 12. ICP-OES Phosphorus (P) Measurements for SRM 956e.

Each symbol denotes the result of one ID-SF-ICP-MS measurement. Horizontal lines bound the mean ± standard deviation intervals.

8.5. Results

The results for all three levels of the SRM 956d control overlap with their non-certified values.

Table 23 lists the P mass concentration and amount concentration values for the three levels of SRM 956e. The relatively large uncertainty associated with Level 3 measurements suggests that P in the level 3 material is at or below the method's limit of quantification. The P results are indicative but not suitable for certification.

Table 23. Phosphorous (P) Mass and Amount Concentrations of SRM 956e.

	mg/dL			mmol/L		
	X_P	$u(x_P)$	$U(x_P)$	C_P	$u(c_P)$	$U(c_P)$
Level 1	13.18	0.11	0.23	4.254	0.036	0.073
Level 2	11.36	0.11	0.23	3.666	0.036	0.073
Level 3	9.53	0.37	0.77	3.08	0.12	0.25

9. Chloride (Cl^-)

Chloride (Cl^-) was determined by microcoulometry, a titration method in which Cl^- was reacted quantitatively with Ag^+ ions that were coulometrically generated at a silver anode. The data were corrected for the presence of other electro-active interferents and bromide (Br^-). The analytical system was qualified by the successful evaluation of an aqueous 0.1 mmol/g potassium chloride (KCl) solution prepared from SRM 999c Potassium Chloride Primary Standard [35].

9.1. Materials

Twelve randomly selected ampoules of SRM 956e at each of the three Levels were analyzed over nine days with two ampoules from two different levels analyzed each day. Three vials of the SRM 909c Frozen Human Serum [36] were analyzed on each of three days as a control. Ampoules contained sufficient serum for two consecutively performed analyses of serum.

The acid reagent was prepared using ACS grade reagents in the following volume fraction composition: 0.9 % polyvinyl alcohol (Sigma), 0.63 % nitric acid (J.T. Baker), 10.5 % acetic acid (Fischer Chemical), 0.00745 % potassium chloride (Sigma-Aldrich), and 88 % 18 MΩ/cm water.

9.2. Sample Preparation

Each ampoule was thawed for 60 min after removal from the freezer. Roughly halfway through the thawing process, ampoules were gently inverted five times to mix the contents. Immediately before it was open, the ampoule was gently inverted an additional five times. The ampoules, which were not scored, required filing prior to opening.

Sample vials were filled with approximately 2.5 mL of acid reagent which was titrated to remove the small amount of chloride. Aliquots of serum were removed from the freshly opened ampoule and delivered gravimetrically to the titration vessel containing the acid reagent using a 100 µL Eppendorf pipette. Each added aliquot was weighed by difference in the pipette tip to a resolution of 10 µg with the aid of a custom-made pipette tip holder. The holder was constructed from 1.6 mm (14 –gauge) Cu wire and a 4 mm length of 3.2 mm (1/8 inch) outer diameter Tygon tubing. The holder retained the pipette tip loosely (without an airtight seal) in a slightly inclined position (tip pointing up) and prevented the aliquot from flowing out of the tip or back towards its base during weighing. Surface tension retained the liquid in the pipette tip. The pipette tip (with sample aliquot or residual sample after delivery) was carefully removed from the pipette and transferred to the holder for each weighing.

Corrections for air buoyancy on mass were based on the density, ρ_{serum}, of each material. The reference density of the stainless-steel calibration weights was 8.0 g/cm^3. Air buoyancy corrections required the equation of state for air density [37].

9.3. Microcoulometric Titrations

For each analysis, nine successive titrations were performed on gravimetric aliquots of the sample serum. The measurement equation for the (uncorrected) microcoulometric titration of Cl^- for each titration is:

$$c_{\mathrm{Cl}} = \frac{I\, t\, \rho_{\mathrm{serum}}}{F\, m_{\mathrm{sample}}} = \frac{I\, r\, \rho_{\mathrm{serum}}}{F\, m_{\mathrm{sample}}\ \mathrm{d}r/\mathrm{d}t} \tag{18}$$

where c_{Cl} is the amount concentration of chloride uncorrected for electroactive interferents or bromide (Br^-) in the serum, I is the constant current, t is the time that I is applied, F is the Faraday constant of 96 485.332 12 C/mol [38], m_{sample} is the mass of the titrated aliquot of sample, r is the counter reading (display), and dr/dt is the counting rate of the counter per s. This equation is based on Faraday's Laws of Electrolysis and the definition of amount concentration. The chloridometer is designed such that the nominal value of (I/(F dr/dt)) as used equals 1×10^{-5} mol/count, corresponding to a nominal value of I of 5.7891 mA.

The chloridometer was calibrated both before and after the SRM 956e chloride analyses. The value of current, I, delivered by the chloridometer was calibrated against the NIST high precision coulometry system. A high-precision 100 Ω standard resistor connected to a high-impedance voltmeter were calibrated as a pair using the known current from the NIST coulometry system. This calibration was performed six times before the analysis. Resistance readings from a secondary standard resistor were taken every second with an average of 3922 readings taken for each calibration against the high-precision resistor. The mean of each set of readings was taken as a single calibration.

The voltmeter – standard resistor pair was used to measure the current generated by the constant-current supply of the chloridometer. This calibration was performed 79 times prior to analysis and 24 times after analysis by initiating "dummy" titrations (without solution). The chloridometer was substituted for the low-current source in the high-precision coulometry system, thus permitting automated triggering of the timer and eliminating the manual timing previously used. Current readings were obtained approximately every 1.2 seconds following the initiation of current flow with an average of around 214 readings taken for each calibration to parallel the approximate period corresponding to the serum titrations. The mean of each set of readings was taken as a single calibration. The recheck of the voltmeter – standard resistor pair vs. the high-precision coulometry system following the measurement of I yielded an identical result to within 0.001 %. Values for dr/dt (counts/s) were simultaneously collected during the current calibrations. The timer automatically stopped when the counter was stopped by lowering the cell holder and count values from the chloridometer were recorded to determine dr/dt for each calibration. Only the calibrations (n = 86) which bracketed the range of counts obtained during the analyses were used to determine dr/dt = 5.999 66 counts/s. The mean of all 22 052 readings were used to estimate c_{Cl}.

Values for constant current for the high-precision coulometry system were obtained from the relevant calibration of the constant-current supply. The experimental value of I/(F dr/dt) 1.000 64 × 10^{-5} mol/count, was obtained from these calibrations and the Faraday constant.

9.4. Correction for Electroactive Impurities in Serum Samples

For each sample, a series of nine successive titrations were performed. Results for the uncorrected c_{Cl} for each set of titrations typically increased slowly with the number of additions (hence, the volume of serum added) in a roughly linear fashion suggesting the presence of an anodically electroactive species in the sample. Extrapolation of the results from the second through the ninth titration were performed for each trial. The intercept and standard deviation of the intercept for the extrapolation were both obtained from the Excel LINEST worksheet function to yield the uncorrected c_{Cl} values for the given trial. These values are listed and summarized in Table 24 and displayed in Fig. 13.

Table 24. Uncorrected Chloride (Cl^-, mmol/L) Measurement Results for SRM 956e.

		Level 1		Level 2		Level 3	
Ampoule	Rep	c_{Cl^-}	$s(c_{Cl^-})$	c_{Cl^-}	$s(c_{Cl^-})$	c_{Cl^-}	$s(c_{Cl^-})$
1	1	98.17	0.61	110.71	0.53	125.24	0.30
	2	98.11	0.58	110.88	0.29	125.29	0.34
2	1	97.90	0.29	110.45	0.56	125.74	0.39
	2	98.00	0.38	110.82	0.37	125.40	0.46
3	1	97.40	0.42	110.98	0.26	125.43	0.79
	2	97.44	0.39	111.12	0.21	125.23	0.81
4	1	98.23	0.90	110.64	0.25	125.31	0.42
	2	97.83	0.23	111.03	0.21	125.24	0.70
5	1	98.31	0.57	111.10	0.57	123.94	0.51
	2	98.66	0.65	111.01	0.14	125.39	0.39
6	1	98.20	0.24	110.73	0.24	125.10	0.22
	2	98.21	0.52	111.15	0.28	125.73	0.50
7	1	97.89	0.13	110.69	0.39	124.85	0.10
	2	98.32	0.25	111.25	0.42	125.21	0.37
8	1	98.48	0.28	111.17	0.29	125.20	0.16
	2	98.23	0.22	111.21	0.40	125.37	0.17
9	1	98.38	0.48	110.98	0.12	125.78	0.57
	2	98.24	0.14	111.09	0.24	124.83	0.44
10	1	98.48	0.70	110.67	0.73	125.14	0.56
	2	98.10	0.31	110.76	0.18	125.19	0.42
11	1	97.84	0.14	111.08	0.43	125.50	0.31
	2	98.05	0.57	111.25	0.15	125.35	0.34
12	1	97.99	0.38	110.42	0.16	125.25	0.15
	2	97.66	0.16	111.15	0.26	125.82	0.20
Mean:		98.09		110.93		125.27	
s_{within}:			0.44		0.37		0.44
$s_{between}$:		0.06		0.05		0.08	

The s_{within} values are pooled standard deviation of the individual standard deviations for each set of titrations. The $s_{between}$ values are the standard deviation of the mean of the entire set of analyses. There was no observed drift in the measurement results over the course of the experiments.

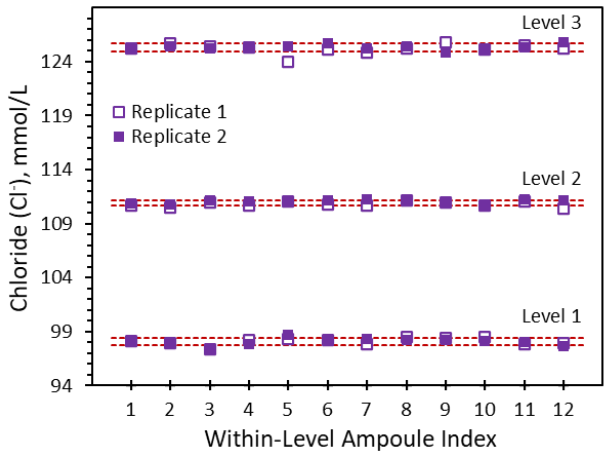

Fig. 13. Uncorrected Chloride (Cl^-) Measurements for SRM 956e.

9.5. Correction for Bromide Mass Fraction

The coulometric method is based on the electrochemical generation of Ag^+, which reacts with Cl^- to form AgCl. Bromide (Br^-), iodide (I^-), and halide-like species (e.g., CN^-) that form precipitates with Ag^+ and have smaller solubility product constants than AgCl, also contribute to the uncorrected c_{Cl}. Based on historical data, only Br^- is expected to be present at amount concentrations larger than the uncertainty in the uncorrected c_{Cl}.

9.5.1. Bromine (Br) by ICP-MS

Inductively coupled plasma mass spectrometry (ICP-MS) was used to determine the mass fraction of bromine (Br) in the SRM 956e materials. While ICP-MS cannot differentiate the chemical forms of Br in the sample, the most stable and likely form of bromine in serum is Br^-.

Three ampoules of each level of SRM 956e and one ampoule of each level of SRM 956d were analyzed as controls. While its Certificate of Analysis (COA) does not provide them, in-house Br values obtained during the SRM 956d certification provided quasi-reference values.

SRM 3184 Bromide Anion (Br-) Standard Solution [39] was used as the calibrant. SRM 3167a Yttrium (Y) Standard Solution [40] was used as an internal standard.

The ICP-MS process was like that described in Section 5.2 but without a collision gas. Br was measured as 79 Da. Y was measured at 89 Da.

Quantitation was by a combination of single-point standard addition (see Section 5.1.4) and external calibration. For each SRM Level, a recovery factor was calculated for the Level as the ratio of the analyte mass fraction obtained from the calibration curve and that obtained by the method of standard addition for the selected sample. The recovery factor at each level of the SRM was applied as a division factor to that level to correct for the mass fraction values obtained from the calibration curve. Table 25 reports and summarizes the results for SRM 956e. Table 26 describes the identified components of uncertainty.

Table 25. ICP-MS Bromine (Br) Mass Fraction Results for SRM 956e, µg/g.

	Level 1	Level 2	Level 3
1	1.292	1.180	1.316
2	1.309	1.150	1.296
3	1.261	1.206	1.289
N:	3	3	3
Mean:	1.288	1.179	1.300
Standard Deviation:	0.024	0.028	0.014
u_{reps} [a]:	0.014	0.016	0.008
u_{blank} [a]:	0.0030	0.0030	0.0030
$u_{calibrant}$ [a]:	0.0015	0.0014	0.0015
u_{purity} [a]:	0.0013	0.0012	0.0014
$u_{weighing}$ [a]:	0.000 12	0.000 11	0.000 12
$U_{linearity}$ [a]:	0.017	0.015	0.017
u [a]:	0.022	0.023	0.019
v [a]:	12	7	60
k [a]:	2.18	2.36	2.00
U [a]:	0.048	0.053	0.038

a) See Table 26 and Eq. 15 for definition of symbols.

Table 26. Components of Bromine (Br) Measurement Uncertainty for SRM 956e.

Component	Basis	Type	v
Sample replication (u_{reps})	Standard uncertainty of replicate ICP-MS measurements of 3 samples	A	2
Blank replication (u_{blk})	Standard uncertainty of replicate ICP-MS measurements of 3 blanks	A	2
Calibrant (u_1)	One-half of the certified 95 % expanded uncertainty of SRM 3184	B	large
Purity (u_2)	Estimated from prior analysis of the materials used to prepare SRM 3184	B	large
Weighing (u_3)	Estimated 0.082 mg weighing uncertainty	B	large
Linearity (u_4)	Standard uncertainty of the slope of the calibration curve multiplied by the sample mean	B	large

9.5.2. Amount Concentrations

Table 27 lists the amount concentration values for Br based on the serum densities from Table 6, the molar mass of Br from Table 7, the mass fraction Br results from Table 25, and the transformations detailed in Eqs. 12 and 13.

Table 27. Bromine (Br) Amount Concentration Results for SRM 956e, mmol/L.

	c_{Br}	$u(c_{Br})$
Level 1	0.016 48	0.000 28
Level 2	0.015 09	0.000 29
Level 3	0.016 64	0.000 24

9.6. Bromide-Corrected Results

The bromide-corrected c_{Cl} are the uncorrected results of Table 24 minus the c_{Br} results of Table 27. Table 28 lists the bromide-corrected mass concentration and amount concentration of chloride in the SRM 956e materials. These results are traceable to the SI by the use a primary coulometric method and correction for all known sources of variability and bias [7].

Table 28. Bromide-Corrected Chloride (Cl^-) Results for SRM 956e.

	mg/dL			mmol/L		
	x_{Cl}	$u(x_{Cl})$	$U(x_{Cl})$	c_{Cl}	$u(c_{Cl})$	$U(c_{Cl})$
Level 1	347.66	0.37	0.74	98.07	0.11	0.21
Level 2	393.19	0.38	0.77	110.91	0.11	0.22
Level 3	444.03	0.47	0.94	125.26	0.14	0.27

The basis for each source of uncertainty in the c_{Cl} values is given in Table 29. The components are listed in order of decreasing contribution to the combined standard uncertainties, $u(c_{Cl})$. The $u(c_{Cl})$ for each Level combines the numerical values of the individual components by differentiation of Eq. 18.

Table 29. Components of Corrected Chloride (Cl^-) Measurement Uncertainty for SRM 956e.

Component	Basis	Type	Distribution
Measurement replication, u_{reps}	Between-ampoule standard deviation	A	Normal
Counter rate, $u(dr/dt)$	(30 to 45) min check vs. timer synchronized with timebase reference. Timer triggered by chloridometer current	B	Uniform
Serum matrix interference, u_{mat}	Historical worst-case nonlinearity of linear extrapolation	B	Normal
Sample delivery, $u(\Delta m_{sample})$	Variability in delivering sample from the pipette tip into the titration vessel	B	Uniform
Mass of sample, $u(m_{sample})$	Mettler balance calibration	B	Combined
Density of serum , $u(\rho_{serum})$	Table 6	B	Combined
Counter reading, $u(r)$	Resolution of counter (0.1 units)	B	Uniform
Deposition of Ag from Ag^+ at cathode, $u(I_{dep})$	Estimated limiting current calculated from amperometric theory	B	Combined
Density of air, $u(\rho_{air})$	1% uncertainty in the density of air from equation of state	B	Uniform
Titration current, $u(I)$	Comparison of chloridometer constant current with constant current of high-precision coulometry system	B	Combined
Br mass fraction, $u(w_{Br})$	Table 27	B	Combined
Faraday constant, $u(F)$	CODATA recommended value	B	Absolute

10. Ionized Calcium (iCa) by ion Selective Electrode (ISE)

Siemens Healthineers Point of Care (Siemens) provided ionized calcium (iCa) measurement results from three Siemens blood gas analysis systems:

- RAPIDLab 1265, a high-volume system for clinical laboratories;
- RAPIDPoint 500e, a benchtop system for critical point-of-care testing; and
- EPOC, a handheld bedside system for rapid turnaround in urgent care situations.

The iCa sensors in all three systems are based on standard potentiometric ion-selective electrode technology. All three systems are calibrated using internal standards traceable to NIST SRMs.

10.1.1. Materials

NIST supplied Siemens with twelve randomly selected ampoules of SRM 956e at each Level and four ampoules of SRM 956d at each level. Siemens supplied all the equipment and supplies required for their analyses.

10.1.2. Sample Analysis

All samples were measured on four days according to the design of Table 30. One measurement was made on each ampoule.

Table 30. Measurement Sequence Design.

	Day 1	Day 2	Day 3	Day 4
1	956d:Lv1:Amp01	956e:Lv1:Amp04	956e:Lv1:Amp07	956e:Lv1:Amp10
2	956e:Lv1:Amp01	956d:Lv1:Amp02	956e:Lv1:Amp08	956e:Lv1:Amp11
3	956e:Lv1:Amp02	956e:Lv1:Amp05	956d:Lv1:Amp03	956e:Lv1:Amp12
4	956e:Lv1:Amp03	956e:Lv1:Amp06	956e:Lv1:Amp09	956d:Lv1:Amp04
5	956e:Lv2:Amp01	956e:Lv2:Amp04	956e:Lv2:Amp07	956d:Lv2:Amp04
6	956d:Lv2:Amp01	956e:Lv2:Amp05	956e:Lv2:Amp08	956e:Lv2:Amp10
7	956e:Lv2:Amp02	956d:Lv2:Amp02	956e:Lv2:Amp09	956e:Lv2:Amp11
8	956e:Lv2:Amp03	956e:Lv2:Amp06	956d:Lv2:Amp03	956e:Lv2:Amp12
9	956e:Lv3:Amp01	956d:Lv3:Amp02	956e:Lv3:Amp07	956e:Lv3:Amp10
10	956e:Lv3:Amp02	956e:Lv3:Amp04	956d:Lv3:Amp03	956e:Lv3:Amp11
11	956e:Lv3:Amp03	956e:Lv3:Amp05	956e:Lv3:Amp08	956d:Lv3:Amp04
12	956d:Lv3:Amp01	956e:Lv3:Amp06	956e:Lv3:Amp09	956e:Lv3:Amp12

Because the pH of the solution affects the iCa levels, the ampoules were thawed and re-equilibrated with the gas in the ampoule headspace using the following procedure [8]:

- Remove samples from freezer and thaw at (20 to 24) °C for 100 min.
- During the first few minutes of thawing, inspect ampoules carefully for cracks or breaks. Appropriately discard ampoules that are cracked or broken.
- After thawing, shake each ampoule vigorously with an up and down motion along the cylindrical axis for 10 seconds to create foam.

- Wait an additional 30 min after shaking, then begin analyzing the samples.
- 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 min of opening the ampoule.
- If it is not possible to aspirate sample directly from the ampoule into the analyzer for the system being used, the sample may be aspirated into a syringe while minimizing contact with air. The sample should be analyzed within one min of opening the ampoule.

The iCa results for the SRM 956e materials are displayed and summarized in Table 31. The results are displayed in Fig. 14 as functions of the ampoule index.

Table 31. Ionized Calcium (iCa, mg/dL) Measurement Results for SRM 956e.

	RapidLab			RapidPoint			Epoc		
	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3
1	6.691	5.472	4.261	6.864	5.432	4.332	7.303	5.875	4.920
2	6.687	5.489	4.233	6.932	5.421	4.311	7.277	6.031	4.669
3	6.715	5.427	4.228	6.797	5.372	4.294	7.175	6.098	4.810
4	a	5.588	4.241	6.891	5.606	4.375	7.298	6.056	4.841
5	a	5.471	4.209	6.862	5.628	4.373	7.440	6.227	a
6	6.804	5.506	4.221	6.904	5.505	4.379	7.619	6.027	4.885
7	6.687	5.383	4.293	6.818	5.348	4.328	7.476	6.032	4.826
8	6.726	5.387	4.259	6.760	5.340	4.332	7.314	6.101	4.890
9	6.817	5.425	4.233	6.770	5.273	4.300	7.417	6.074	4.775
10	6.566	5.513	4.269	6.859	5.674	4.343	7.435	6.245	4.935
11	6.760	5.369	4.261	6.847	5.567	4.350	7.425	6.240	4.843
12	6.757	5.385	4.248	6.910	5.455	4.357	7.486	6.135	4.765
N:	10	12	12	12	12	12	12	12	11
Mean:	6.721	5.451	4.246	6.851	5.468	4.340	7.389	6.095	4.833
Standard Deviation:	0.072	0.067	0.023	0.055	0.128	0.029	0.119	0.107	0.077

a) Sample lost during analysis

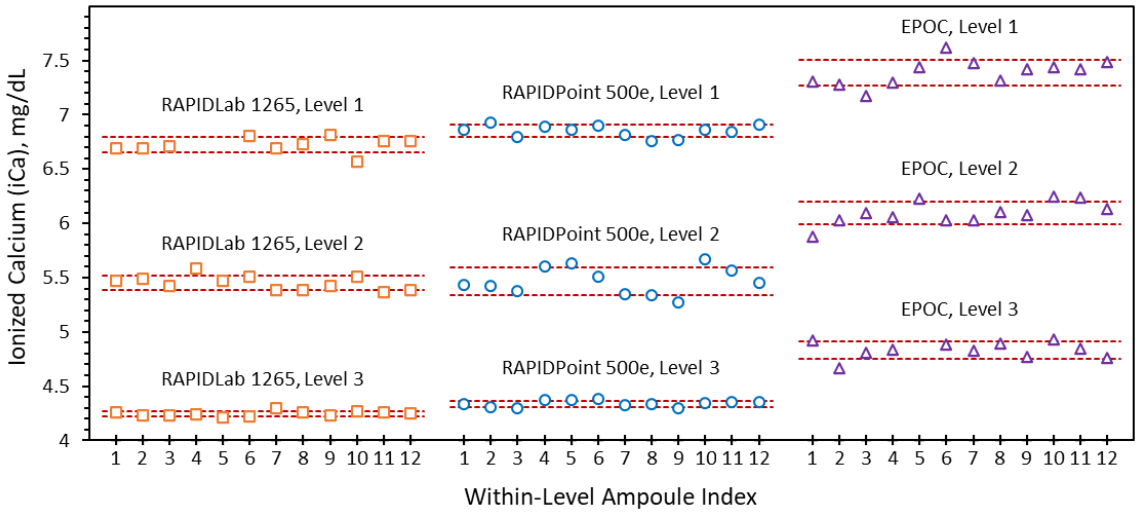

Fig. 14. Ionized Calcium (iCa, mg/dL) Measurements for SRM 956e.

Each symbol denotes the result of one measurement of one ampoule. Dashed horizontal lines bound the mean ± standard deviation intervals.

10.1.3. Consensus

The consensus results for iCa in SRM 956e Levels 1 to 3 using the multi-method analysis described in Section 4.2 are depicted in Fig. 15. Table 32 lists the mass concentration values; it also presents amount concentration values, derived using Eq. 13 and the Ca molar mass listed in Table 7. While the three ISE methods were calibrated with CRMs, they are not necessarily independent and there are uncompensated biases. While indicative, they are not suitable for certification.

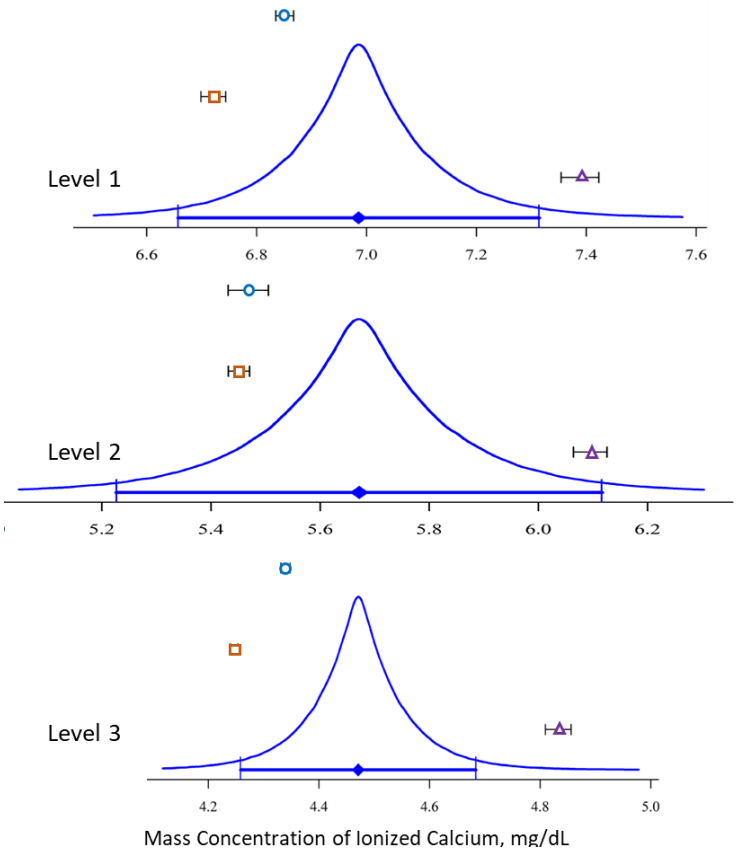

Fig. 15. Consensus Ionized Calcium Mass Concentration Results for SRM 956e.

Open squares with error bars represent the mean RAPIDLab results with their associated standard uncertainties, open circles with error bars represent the RAPIDPoint results, and open triangles with error bars represent the EPOC results. Curves represent the empirical distribution of consensus values. Solid diamonds with bars represent the final consensus values with their associated 95% level of confidence symmetric coverage intervals.

Table 32. Ionized Calcium (iCa) Mass and Amount Concentrations in SRM 956e.

	mg/dL			mmol/L		
	x_{iCa}	$u(x_{iCa})$	$U(x_{iCa})$	c_{Ca}	$u(c_{iCa})$	$U(c_{iCa})$
Level 1	6.986	0.155	0.329	1.743	0.039	0.082
Level 2	5.672	0.210	0.445	1.415	0.052	0.111
Level 3	4.471	0.101	0.213	1.116	0.025	0.053

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Appendix A. List of Symbols, Abbreviations, and Acronyms

A.1. Symbols

$c_{\text{subscript}}$	amount concentration of a substance
$s_{(\cdot)}$ or $s_{\text{subscript}}$	standard deviation of a quantity
$u_{(\cdot)}$ or $u_{\text{subscript}}$	standard uncertainty of a quantity
$U_{(\cdot)}$ or $U_{\text{subscript}}$	expanded uncertainty of a quantity
$w_{\text{subscript}}$	mass fraction of a substance
$x_{\text{subscript}}$	mass concentration of a substance
ρ	density

A.2. Acronyms

ACS	American Chemical Society
CLSI	Clinical Laboratory Standards Institute
COA	Certificate of Analysis
CRM	certified reference material
iCa	ionized calcium
ICP-OES	inductively coupled plasma optical emission spectrometry
ICP-MS	inductively coupled plasma mass spectrometry
ID-ICP-MS	isotope dilution inductively coupled plasma mass spectrometry
ID-SF-ICP-MS	isotope dilution sector field inductively coupled plasma mass spectrometry
ISE	ion-selective electrode
IRB	Institutional Review Board
LDPE	low-density polyethylene
NIST	National Institute of Standards and Technology
ORM	NIST Office of Reference Materials
RSD	relative standard deviation
ROA	report of analysis
SAS	“standard as sample” control materials
SI	International System of Units (Système international d'unités)
SOR	Statement of Requirements
SRM	Standard Reference Material
TPOC	Technical Point of Contact