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Certification of Standard Reference Material® 967b Creatinine in Frozen Human Serum

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Blaza Toman

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Abstract

Standard Reference Material (SRM) 967b Creatinine in Frozen Human Serum is intended for use in validating creatinine measurement procedures used by *in vitro* diagnostics manufacturers, reference laboratories, and clinical laboratories in the United States and globally. A unit of SRM 967b consists of two 1 mL vials each of two levels of creatinine for a total of four vials per unit. This publication documents the production, analytical methods, and computations involved in characterizing this product.

Keywords

Creatinine; Isotope Dilution Liquid Chromatography Mass Spectrometry (ID-LC-MS); Reference Measurement Procedure (RMP); Standard Reference Material (SRM).

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1. Introduction

Creatinine is an easily measured byproduct of muscle metabolism that is excreted unchanged by the kidneys. If the filtration in the kidney is deficient, blood creatinine concentrations rise. Accurate measurement of creatinine concentration in serum provides clinically important estimates of kidney function [1,2]. The tautomeric chemical structure of creatinine is depicted in Fig. 1.

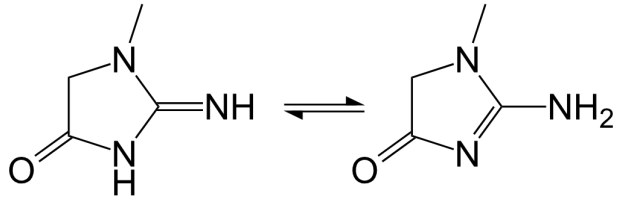

Fig. 1. Chemical structure of Creatinine.

The National Institute of Standards and Technology (NIST) Standard Reference Material® (SRM®) 967b Creatinine in Frozen Human Serum is intended to support serum creatinine measurement processes of *in vitro* diagnostics manufacturers, reference laboratories, and clinical laboratories in the United States and globally. SRM 967b is the third member of the SRM 967 series. The original SRM 967 [3] was issued in 2007 with the last unit sold in July of 2009. It was followed by SRM 967a [4] in January 2010 with the last unit sold in October of 2021. The sales history of SRM 967 and SRM 967a as a function of calendar year is displayed in Fig. 2. The proportions of their sales to customers in the US, Canada, Europe, Asia, and the rest of the world are displayed in Fig. 3.

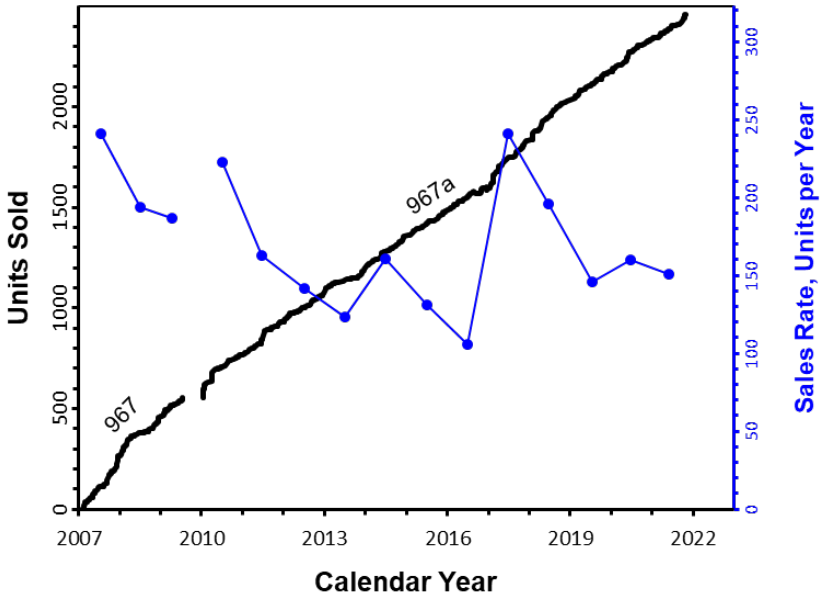

Fig. 2. Sales History of SRM 967, 2007 to 2022.

The thick black line depicts the cumulative distribution of sales as a function of the order date; it is plotted using the "Units Sold" axis at the left of the plot. The thin blue line depicts the total units sold per year; it is plotted using the "Sales Rate, Units per Year" axis to the right of the graph.

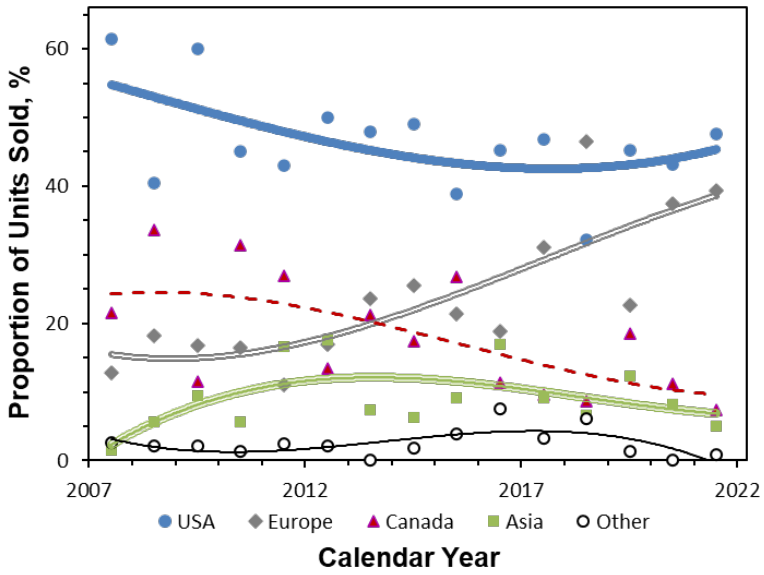

Fig. 3. Location of Customers for SRMs 967 and 967a.

The solid circles and the thick trendline display the proportion of sales to customers within the USA from the first sale in February of 2007 to date of the last unit sold in October of 2021. Solid diamonds and the double-line trendline display the proportion of units sold to customers in Europe (including the United Kingdom) customers; solid triangles and the dashed line display the proportion sold to customers in Canada, solid squares and the triple-line trendline display the proportion solid to customers in Asia. The open circles and thin single-line trendline display the proportion of units sold to customers elsewhere.

A unit of SRM 967b consists of two vials each of SRM 967b Creatinine in Frozen Human Serum (Low Level) and SRM 967b Creatinine in Frozen Human Serum (High Level). Each of the four vials contain approximately 1.1 mL of human serum. The creatinine concentration in these two materials was assigned using NIST's isotope-dilution liquid chromatography mass spectrometry (ID-LC-MS) method [5,6]. This method is recognized by the Joint Committee for Traceability in Laboratory Medicine (JCTLM) as a reference measurement procedure (RMP) [7]. The RMP was validated on the current liquid chromatography-mass spectrometry (LC-MS) system, with the only modification being that both calibrants and samples be diluted 1-to-50 by volume.

The uncertainty of creatinine values assigned using the NIST RMP has been proven fit-for-purpose. Metrological traceability to the International System of Units (SI) of the assigned creatinine values in the SRM 967b materials is through calibration with SRM 914b Creatinine [8]. Based on experience with SRMs 967 and 967a, the creatinine levels of the SRM 967b materials are expected to be stable for at least seven years post-production. Stability will be evaluated within one year of the expiration date, with intermittent monitoring through its use as a control whenever creatinine in serum measurements are performed within NIST.

2. Production

A contract was awarded to Solomon Park Research Laboratories, Burien WA 98148, for the preparation of 4,000 vials each of SRM 967b Creatinine in Frozen Human Serum (Low Level) and SRM 967b Creatinine in Frozen Human Serum (High Level). The High Level material was to have a creatinine concentration of (4.0 ± 0.2) mg/dL to be achieved by spiking high-purity creatinine into a normal human serum pool. The Low Level material was to have a creatinine concentration of (0.4 ± 0.05) mg/dL to be achieved using only native serum.

The target range for the Low Level could not be achieved due to the difficulty in obtaining a sufficient volume of serum with low native creatinine concentration. After representatives from Solomon Park and NIST discussed available options, NIST agreed to accept a Low Level material with a screening value of 0.64 mg/dL. With this exception, the SRM 967b materials were acquired and produced as described in the following extracts from the Statement of Work developed for this SRM renewal.

2.1. Scope of Work

The contractor shall produce 4,000 vials each of two serum materials at the creatinine concentrations specified below, with each vial containing (1.1 ± 0.1) mL of serum. The contractor shall acquire a minimum of 4,000 mL of serum pooled at each level according to the updated CLSI C37-A protocol for preparation of commutable pooled serum reference materials [9,10]. The pool of serum for the low level must not be diluted or stripped to achieve the target (0.4 ± .05) mg/dL creatinine. The low-level serum pool must be discreet from the high-level pool. A separate, normal human serum pool must be spiked with crystalline creatinine to achieve an elevated level of creatinine (4.0 mg/dL ± 0.2 mg/dL). The serum shall be aliquoted into vials that are capable of withstanding long-term storage (5 to 10 years) at -80 °C.

2.2. Specific Tasks and Requirements

Task 1: Low Level: Contractor shall provide 4,000 vials containing (1.1 ± 0.1) mL pooled human serum containing (0.4 ± 0.05) mg/dL creatinine

Task 2: High Level: Contractor shall provide 4,000 vials containing (1.1 ± 0.1) mL pooled human serum containing (4.0 ± 0.2) mg/dL creatinine

The High Level must be prepared by augmenting a normal serum pool (with a starting creatinine level of approximately 0.6 mg/dL to 1.2 mg/dL) with crystalline creatinine. The contractor is responsible for obtaining the creatinine used to spike the High Level.

Task 3: Vial stress test results in accordance with the following and the requirements herein.

The contractor shall provide the NIST Technical Point of Contact (TPOC) with vial stress test results prior to filling and the Certificate of Analysis, including pilot/validation results, prior to shipping of the vials to NIST.

Task 4: Certificate of Analysis, including pilot/validation study results in accordance with the requirements herein.

Task 5: Material Preparation Detail Report

The contractor shall provide the TPOC with the material preparation report, including the source of the added creatinine (brand, catalog number, and lot) and any deviations from the specified standard, and the Biosafety Report not later than 180 days after award of this requirement to ensure documentation of any deviations as well as to provide information that shall be utilized in the NIST material acquisition Report of Analysis.

This information must be provided to the TPOC as a pdf file, via email.

Task 6: Biosafety Report in accordance with the requirements herein.

2.3. Task Requirements

The contractor shall test the serum units for biosafety. All sera shall be demonstrated to be non-reactive when tested for hepatitis B surface antigen, hepatitis C virus, human immunodeficiency virus, and human immunodeficiency virus antigen 1 by tests licensed by the U.S. Food and Drug Administration.

The contractor shall provide a written Biosafety Report stating the negative results of all single-donor serum units utilized for SRM 967b preparation. Donor units testing positive for any of the stated infectious agents must not be included in the serum pools. Personal identifiers of donors must not be disclosed to NIST. For the purpose of this statement of work, “Donor units” are serum volumes collected from individual human donors. The contractor shall draw samples or obtain samples from a secondary blood collection site that follows the stated guidelines.

After the donor units are selected, they must be pooled. The contractor shall thoroughly blend and filter the pools (0.22 μm PVDF) as defined in CLSI C37-A [9,10]. The contractor must dispense the serum into labeled amber glass serum bottles capable of withstanding ultracold temperatures (-80 °C), each containing nominally 1 mL of serum (1.1 mL dispensed with an accuracy better than 0.1 mL), and sealed under nitrogen with a butyl rubber stopper and an aluminum crimp cap. The vials must be 3 mL amber serum vials, (17 x 37) mm, USP Type 1 borosilicate glass, 13 mm crimp finish, available from Voigt Global Distribution (catalogue #62413PV-3) or equivalent.

The contractor must stress test the lot of serum vials prior to filling by picking 10 vials randomly from the lot and subjecting them to 5 freeze and thaw cycles at -80 °C to ensure that there is no breakage, including small cracks or fissures. The contractor must provide the results of the stress test, via email, to the TPOC prior to moving forward with vial filling.

Prior to filling, the contractor shall label vials with labels that are appropriate for use at low temperature; these labels will be provided to the contractor by NIST within 60 days after award.

The contractor must use two different color aluminum crimp caps to differentiate between the two specified serums for easy identification. Vials must be transferred, in fill order, from the bottling equipment to a box with vial dividers in a "Z" pattern, filling each row left to right. The location of the first vial in each box shall be noted on each side of the outside corner of the box, and boxes will be numbered sequentially. Boxes shall be labeled to indicate their contents.

Materials shall be stored frozen (-80 degrees °C) prior to overnight shipment on dry ice to NIST. Overnight shipments must not be sent on Friday/Saturday or before a federal holiday. In addition, the contractor must notify the TPOC at least 48 hours in advance of the shipment to be sure that NIST staff are available to receive the shipment. Delivery must be completed not later

than 180 days after award. The contractor shall notify the NIST TPOC, by e-mail, providing the date the deliverables are shipped, shipping method, tracking number, method of delivery and the anticipated delivery date it will arrive at NIST.

The contractor shall assess homogeneity after vial filling and prior to shipment of vials to NIST, as defined in CLSI C37-A (Appendix A, page 9) [9,10] measuring creatinine with an enzymatic-based assay instead of cholesterol. The method used to assay creatinine must have a within-run coefficient of variation (CV) < 2 % for creatinine values of 0.8 mg/dL and higher. The CLSI document includes statistics and acceptance criteria.

The contractor shall follow the guidelines set forth for base pool specifications as detailed in CLSI C37-A [9,10] for the preparation of this two-level creatinine in serum material. The target creatinine concentration levels are given in the Specific Tasks above. The low-level material must be based only on pooling of serum and must not be diluted or stripped to obtain the target creatinine level. The high-level material must be prepared by spiking a normal serum pool with crystalline creatinine. The serum shall be filled into pre-labeled vials, flushed and sealed under an inert gas, and frozen according to the specifications given in CLSI Document C37-A [9,10].

A pilot/validation run shall be performed by the contractor after vial filling and prior to shipment of the vials to NIST. The contractor shall provide NIST with a Certificate of Analysis indicating creatinine concentrations of individual vials by an enzymatic clinical method from vials selected by the contractor from each level of material according to CLSI C37-A [9,10]. This data must be provided by NIST as a pdf via email to the TPOC before shipment of the entire production lot.

2.4. Deliverables and Deliverable Due Dates

Biosafety Report: Due 48 hours prior to shipment

Vial Stress Test Results: Due prior to vial filling

Material Preparation Detail Report: Due 48 hours prior to shipment

Notification of Shipment: Due 48 hours prior to shipment

Certificate of Analysis: Due 48 hours prior to shipment

Low Level SRM – 4000 vials: Due 180 days from the date of award

High Level SRM – 4000 vials: Due 180 days from the date of award

2.5. Protection of Human Subjects

The contractor is required to draw the serum specimens or obtain serum specimens from an appropriate source. Human Subjects Clearance may be required. The contractor shall be responsible to satisfy all human subjects requirements prior to performing work relevant to this clearance. Reference the following requirements as specified in the Code of Federal Regulations 1352.235-70 Protection of human subjects.

(a) Research involving human subjects is not permitted under this award unless expressly authorized in writing by the Contracting Officer. Such authorization will specify the details of the approved research involving human subjects and will be incorporated by reference into this contract.

(b) The Federal Policy for the Protection of Human Subjects (the "Common Rule"), adopted by the Department of Commerce at 15 CFR part 27, requires contractors to maintain appropriate policies and procedures for the protection of human subjects in research. The Common Rule defines a "human subject" as a living individual about whom an investigator conducting research obtains data through intervention or interaction with the individual, or identifiable private information. The term "research" means a systematic investigation, including research development, testing and evaluation, designed to develop or contribute to generalizable knowledge. The Common Rule also sets forth categories of research that may be considered exempt from 15 CFR part 27. These categories may be found at 15 CFR 27.101(b).

(c) In the event that human subjects research involves pregnant women, prisoners, or children, the contractor is also required to follow the guidelines set forth at 45 CFR part 46 subpart B, C and D, as appropriate, for the protection of members of a protected class.

(d) Should research involving human subjects be included in the proposal, prior to issuance of an award, the contractor shall submit the following documentation to the Contracting Officer:

(1) Documentation to verify that contractor has established a relationship with an appropriate Institutional Review Board ("cognizant IRB"). An appropriate IRB is one that is located within the United States and within the community in which the human subjects research will be conducted;

(2) Documentation to verify that the cognizant IRB possesses a valid registration with the United States Department of Health and Human Services' Office for Human Research Protections ("OHRP");

(3) Documentation to verify that contractor has a valid Federal-wide Assurance (FWA) issued by OHRP.

(e) Prior to starting any research involving human subjects, the contractor shall submit appropriate documentation to the Contracting Officer for institutional review and approval. This documentation may include:

(1) Copies of the human subjects research protocol, all questionnaires, surveys, advertisements, and informed consent forms approved by the cognizant IRB;

(2) Documentation of approval for the human subjects research protocol, questionnaires, surveys, advertisements, and informed consent forms by the cognizant IRB;

(3) Documentation of continuing IRB approval by the cognizant IRB at appropriate intervals as designated by the IRB, but not less than annually; and/or

(4) Documentation to support an exemption for the project from the Common Rule [Note: this option is not available for activities that fall under 45 CFR part 46 subpart C].

(f) In addition, if the contractor modifies a human subjects research protocol, questionnaire, survey, advertisement, or informed consent form approved by the cognizant IRB, the contractor shall submit a copy of all modified material along with documentation of approval for said modification by the cognizant IRB to the Contracting Officer for institutional review and approval. The contractor shall not implement any IRB approved-modification without written approval by the Contracting Officer.

(g) No work involving human subjects may be undertaken, conducted, or costs incurred and/or charged to the project, until the Contracting Officer approves the required appropriate documentation in writing.

2.6. Acceptable Quality Level

The vials of all pooled serum with the associated physical and chemical properties specified in this Statement of Work must be suitable for use as reference materials. If deficiencies or inconsistencies between the material and the documentation listed in the Specific Tasks and Requirements are found, or, if less than the stated number of vials are received intact, the contractor shall have 30 days from notification by the TPOC to correct the deficiency at no additional cost to the Government.

2.7. Monitoring Method

The NIST TPOC will verify that the materials meet all requirements of the statement of work. Acceptance will be based upon the delivery of 4,000 vials of each pooled serum material (8,000 vials total) intact and unbroken, accompanied by screening results for creatinine which indicate that the two levels of material possess creatinine levels within the specified target ranges. Verification and acceptance will be completed no later than 30 days after receipt of the vials at NIST.

For the purpose of this statement of work, verification shall consist of physically inspecting vials to ensure they are intact and reviewing the pilot analysis results provided by the contractor.

2.8. Minimum Contractor Qualifications

- Documented compliance with ISO 13485:2016 “Medical devices -- Quality Management Systems -- Requirements for Regulatory Purposes”. Documentation may be a certificate from ISO or a certificate from a certification organization.
- Experience in analyzing creatinine in human serum.

2.9. Rights in Data

The Government will retain unlimited data rights to all required deliverables in accordance with the data rights clause, incorporated by reference, herein.

2.10. Contractor's Certificate of Analysis

SOLOMONPARK
Improving the health of the Human Race

CERTIFICATE OF ANALYSIS

Client:	**NIST**
Product:	**Creatinine 1-2 SRM 967b**
Lot Number:	**4310**
Production Date:	**Level 1: 01/12/2022 (high creatinine)** **Level 2: 06/09/2022 (low creatinine)**
Expiration Date:	**Level 1: 01/12/2026 (high creatinine)** **Level 2: 06/09/2026 (low creatinine)**
Source:	**Frozen Human Serum**
Form:	**4000 x 1.1 mL per pool**
Final Values:	**Level 1: 3.9 mg/dL** **Level 2: 0.64 mg/dL**
Preservative:	**None**
Storage:	**Keep at –70°C**
Shipping Temperature:	**Frozen (on dry ice)**

Tests performed on original donor units using Bio-Rad PR4100 plate reader and reagents from BioRad were non- reactive for HBsAg, HCV, HIV 1 (Groups M and O) and HIV 2. All materials consisting of, containing, or isolated from human sources should be considered potentially hazardous and handled with appropriate precautions. For In Vitro diagnostic use only.

Solomon Park Research Laboratories tel. 425.650.2000
658 S. 152nd St. Burien WA 98148 - email: info@solomon.org

3. Determination of Target Values

An accurate estimate of analyte concentration helps to minimize the uncertainty in ID-LC-MS measurements. Estimates for the creatinine mass fraction, expressed as microgram creatinine per gram serum (µg/g), in the SRM 967b materials were determined using the NIST RMP.

3.1. Materials

SRM 914b Creatinine [8] with a certified mass purity of (99.9 ± 0.1) % was used as the reference standard for creatinine. The internal standard was creatinine-d_3 purchased from Cayman Chemical (Item:16763 Batch: 0533175-17). Ethyl alcohol (200 proof, anhydrous) was purchased from The Warner-Graham Company. Ammonium acetate (99.99 %, Part 43131-1, 02410TS) was purchased from Sigma-Aldrich. High-Performance Liquid-Chromatography (HPLC)-grade water and HPLC-grade acetonitrile were used in the calibration and sample preparation steps, as well as for preparation of LC-MS mobile phases.

SRM 1950 Metabolites in Frozen Human Plasma [11] was used as the control material. Two vials of SRM 1950 were retrieved from -80 °C storage. SRM 1950 is certified for creatinine at a mass concentration of (0.6789 ± 0.0108) mg/dL. Using the provided density value of 1.02086 g/mL, the mass fraction of creatinine in SRM 1950 was determined to be (6.65 ± 0.106) µg/g.

Three vials each of the SRM 967b Low and High Levels were selected for analysis. Vials were selected from the beginning (box 1), middle (box 40), and end (box 81) of the production runs.

3.2. Material Preparation

3.2.1. Reagent Solution

Mobile phase A was 10 mmol/L ammonium acetate solution. The solution was prepared by weighing 0.772 g of ammonium acetate into a 1 L volumetric flask. HPLC grade water was added, swirled to dissolve, and then brought to the mark with additional HPLC-grade water. A 50:50 (volume fraction) acetonitrile/water solution was prepared as mobile phase B for column and instrument cleaning purposes.

3.2.2. Calibrant and Internal Standard

Four independent creatinine stock solutions were prepared by accurately weighing approximately 1 mg of neat SRM 914b Creatinine into an aluminum weighing cup on a Mettler Toledo UMX5. The aluminum weighing cup with weighed creatinine material was then transferred into a 100 mL volumetric flask. The mass of creatinine and flask was tared on a Mettler Toledo XPE205 analytical balance before the addition of 80 mL of HPLC-grade water. The mass of water added was recorded. The stock solution was swirled to make sure the solid was dissolved, and then poured into 150 mL beaker before dividing into 2 mL aliquots and stored in 2 mL capped Eppendorf tubes at -20 °C.

The internal standard stock solution was prepared in the same manner. Approximately 1 mg of creatinine-d_3 was weighed in an aluminum weight cup, transferred to a 100-mL volumetric flask,

dissolved into 80 mL of HPLC-grade water, and then divided into 2-mL aliquots and stored in 2-mL Eppendorf tubes at -20 °C.

Prior to preparing calibrant mixtures, stock solutions were removed from -20 °C storage and allowed to thaw at room temperature. Creatinine calibrants were prepared by adding the varying amounts of creatinine and internal standard solutions to achieve mass ratios of 0.8 to 1.2. All weights were recorded for the accurate determination of mass fraction calculations.

Each creatinine calibrant was further diluted 1 to 50 (volume fraction) where 50 µL of calibrant was added into 2.45 mL of water. Approximately 1 mL of each diluted calibrant was transferred into an HPLC vial for ID-LC-MS analysis.

3.2.3. Samples

Aliquots of the SRM 1950 and SRM 967b samples were spiked with internal standard solution to achieve a mass ratio of 1-to-1 (creatinine-to-creatinine-d_3) according to the following procedure. A 175 µL aliquot of internal standard solution (calculated to be 2.4 µg of creatinine-d_3) was added gravimetrically to a 15 mL conical centrifuge tube. An aliquot (calculated to contain 2.4 µg of creatinine) of thawed plasma or serum was then added by mass. All masses were recorded. The samples were vortex mixed and allowed to equilibrate overnight at 2 °C to 8 °C. Once equilibrated, a volume equal to three times the original volume of plasma or serum plus internal standard of cold ethanol was added to precipitate the proteins. Each sample was then vortex mixed gently and allowed to sit for 5 min at room temperature. Samples were centrifuged in an Eppendorf Centrifuge 5810R at 314.2 rad/s (3000 rpm) for 20 min at room temperature.

The supernatants from each tube were transferred to 4 mL glass vials and dried to completion under gentle nitrogen gas flow in a Thermo Scientific Reacti-Therm III heating module and Reacti-Vap III Evaporation Unit at 40 °C. Samples were reconstituted with 500 µL of HPLC-grade water, vortex mixed, and filtered through a 0.45 µm polyvinylidene difluoride (PVDF) syringe filter into 2 mL Eppendorf tubes.

Each creatinine sample was further diluted 1 to 50 (volume fraction) where 50 µL of filtered sample was added into 2.45 mL of water. Approximately 1 mL of the diluted sample was transferred into an HPLC vial for ID-LC-MS analysis. The remaining reconstituted sample volumes were stored at -20 °C for potential future measurements.

3.3. Instrumental Method

3.3.1. Equipment

All analyses were performed on an Agilent 1200 Series LC system with an Agilent 6130 LC-MS. This instrument was equipped with a binary pump, degasser, autosampler, column compartment, and a single quadrupole MS with electrospray ionization. The instrument was controlled using ChemStation software (Agilent). Separation was achieved on a Luna C18(2) analytical column 25 cm by 4.6 mm, 5 µm particles, purchased from Phenomenex (Torrance, CA).

The separation was isocratic using 100 % mobile phase A at a 0.5 mL/min flow rate for 30 min with a column temperature of 25 °C. The sample injection volume was held constant at 5 µL. Detection was achieved using a single quadrupole MS with electrospray ionization in positive

mode operated in selected ion monitoring (SIM) mode to detect *m*/*z* 114 (creatinine) and *m*/*z* 117 (creatinine-d_3) at fragmentor of 90 V, gain of 1, dwell time of 195 ms. The MS parameters were: drying gas of 12 L/min, nebulizer pressure at 345 kPa (50 psig), voltage at 1500 V, and gas temperature at 350 °C.

3.3.2. Quantitation

The mass fraction of creatinine in SRM 1950 and SRM 967b was determined using an internal standard approach. Two sets of calibrants, control, and samples were prepared as described in above sections with all materials in a set prepared on the same day.

Each calibrant was injected in triplicate, followed by a water blank injection, and then each control and sample were injected in triplicate. The SIM peaks at *m*/*z* 114 and 117 were manually integrated and peak areas recorded.

The response factor (RF) was calculated for each calibrant injection:

$$R = \frac{A_{\text{analyte}}}{A_{\text{IS}}} \frac{m_{\text{IS}}}{m_{\text{analyte}}} \quad (1)$$

Where R is the RF, A_{analyte} is the area of the *m*/*z* 114 (creatinine) peak, A_{IS} is the area of the *m*/*z* 117 (creatinine-d_3) peak, m_{analyte} is the mass of creatinine in the calibrant, and m_{IS} is the mass of creatinine-d_3 in the calibrant.

The average response factor, $\bar{R}$ determined from multiple injections of multiple calibrants, was used to calculate the mass of creatinine in each of the samples:

$$m_{\text{analyte in sample}} = \frac{A_{\text{analyte}}}{A_{\text{IS}}} \frac{m_{\text{IS}}}{\bar{R}} \quad (2)$$

The mass fraction of creatinine in each sample was then calculated:

$$w_{\text{analyte}} = \frac{m_{\text{analyte in sample}}}{m_{\text{sample}}} \quad (3)$$

where m_{sample} is the mass of the sample and w_{analyte} is the estimated mass fraction of creatinine in that sample.

3.3.3. Peak Area Determinations

The *m*/*z* 114 (creatinine) and *m*/*z* 117 (creatinine-d_3) SIM peaks for all calibrant, control, and sample solutions were on-scale, adequately symmetric, with a flat baseline. Representative chromatograms for calibrants, controls, SRM 967b Low Level, and SRM 976b High Level are displayed in Fig. 4.

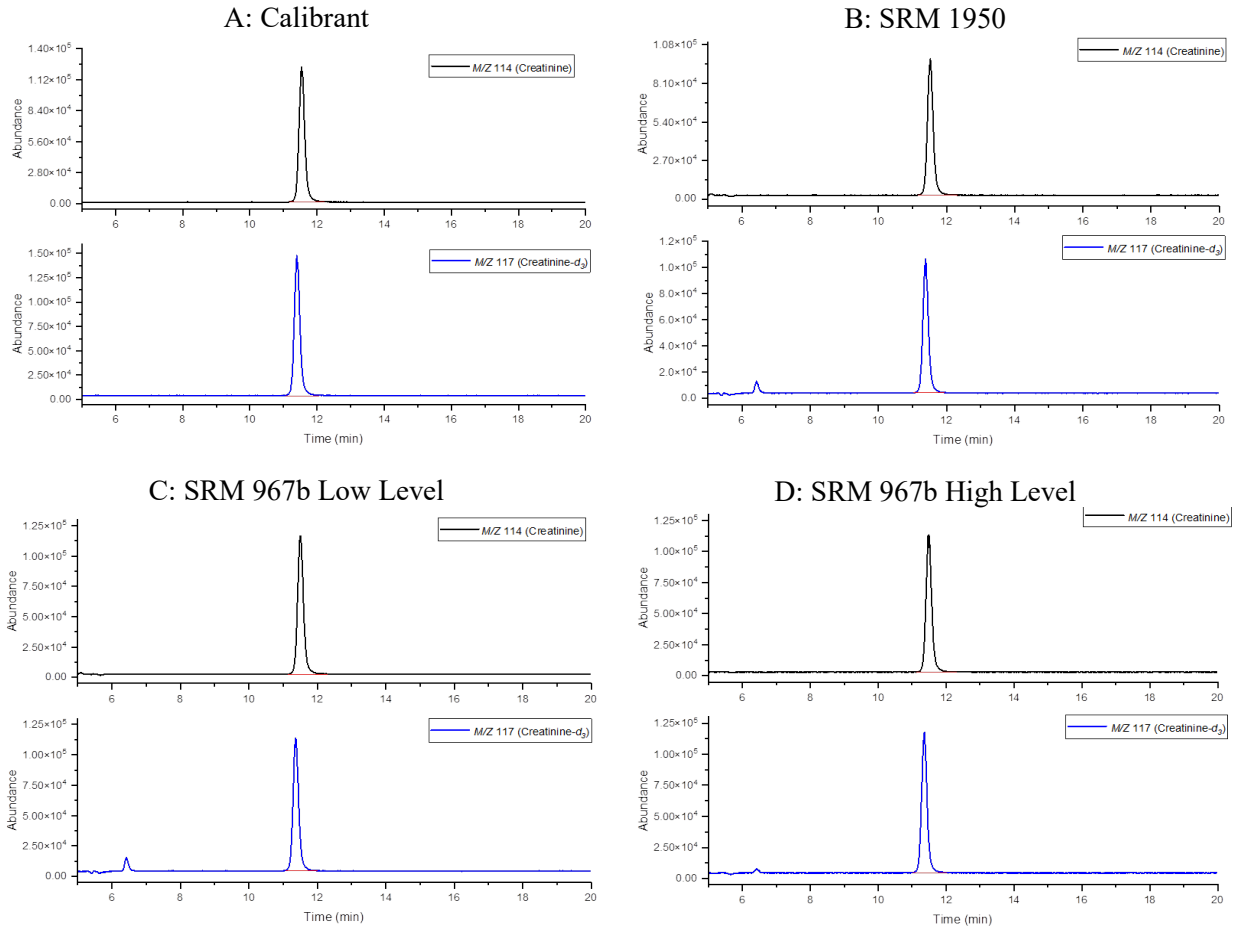

Fig. 4. Representative Target Assessment SIM Chromatograms.

Each panel displays SIM chromatograms at *m/z* 114 (upper sub-panel) and *m/z* 117 (lower sub panel) of one injection: A) calibrant, B) SRM 1950, C) SRM 967b Low Level, and D) SRM 967b High Level.

3.4. Results

Table 1 lists the RFs for the two sets of calibrant, control, and sample solutions. The mean ± standard deviations (SDs) for the two sets are 0.961 ± 0.013 and 0.949 ± 0.011, giving coefficients of variation (CV, also called the "relative standard deviation", expressed as a percentage) of 1.4 % and 1.14 %. These results indicate that the preparation of calibrants was consistent, the calibrants were stable, and that the peak area ratios were repeatable and reproducible over the experimental period.

Table 1. Response Factors for Determination of Target Creatinine Concentrations

Calibrant	Injection	Set 1	Set 2
1	1	0.9811	0.9515
	2	0.9803	0.9451
	3	0.9799	0.9531
2	1	0.9560	0.9326
	2	0.9617	0.9322
	3	0.9605	0.9363
3	1	0.9527	0.9611
	2	0.9520	0.9526
	3	0.9439	0.9510
4	1	0.9561	0.9582
	2	0.9478	0.9619
	3	0.9623	0.9554

Table 2 lists the estimated creatinine mass fractions for the SRM 1950 control material. Sample aliquots of two vials of the control were analyzed within each set; however, the result for the second vial in the second set was invalid due to a weighing error. The mean ± SD of the nine results is (6.683 ± 0.081) µg/g creatinine. The CV of 1.21 % agrees well with the ≤ 1.4 % of the RFs.

Table 2. Creatinine Mass Fraction Estimates for SRM 1950

Set	Vial	Injection	Creatinine, µg/g
1	1	1	6.558
		2	6.647
		3	6.587
	2	1	6.744
		2	6.661
		3	6.667
2	1	1	6.753
		2	6.809
		3	6.720

SRM 1950 is certified for creatinine at a mass concentration of (0.6789 ±0.0108) mg/dL. Using the density value of 1.02086 g/mL provided on the Certificate of Analysis [11] and the following relationship,

$$w_{\text{analyte}}\,\frac{\text{µg analyte}}{\text{g serum}} = x_{\text{analyte}}\,\frac{\text{mg analyte}}{\text{dL serum}} \Big/ \left(\left(\rho\,\frac{\text{g serum}}{\text{mL serum}}\right)\left(\frac{\text{100 mL serum}}{\text{dL serum}}\right)\left(\frac{\text{mg analyte}}{\text{1000 µg analye}}\right)\right) \qquad (4)$$

the creatinine mass fraction is (6.650 ± 0.106) µg/g. The results are plotted in sequential injection order in Fig. 5. The results for the vial 1 aliquot evaluated in set 1 do not overlap those for the vial 1 aliquot evaluated in set 2, suggesting that the measurement process was not in complete control. However, eight of the nine results are withing the certified interval and the mean result is in good agreement with the certified value, suggesting that the process provides accurate estimates of creatinine mass fraction for the SRM 967b materials.

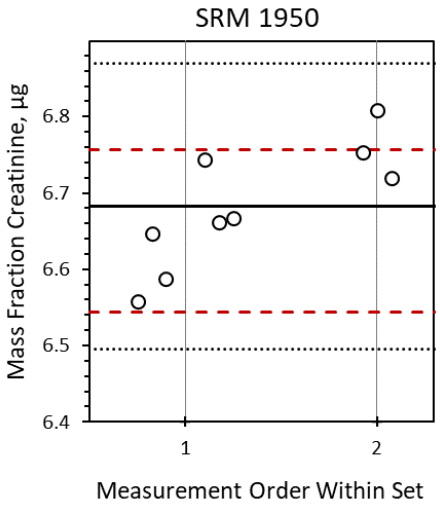

Fig. 5. SRM 1950 Control Measurements.

SRM 1950 results are plotted as a function of measurement order within each set. The open circles represent the measurement results for each injection. The solid horizontal lines represent the mean of all results; the horizontal dotted lines bound the approximate 95 % level of confidence interval about that mean. The horizontal dashed lines represent the 95 % level of confidence certified intervals for the SRM 1950 creatinine value converted from mass concentration to mass fraction. The thin vertical lines indicate measurement set.

Table 3 lists the estimated creatinine mass fractions for the SRM 967b Low Level and High Level materials. Sample aliquots of three vials were analyzed in each set, two vials of one of the two levels and one of the other. The mean ± SD for the two Levels are (6.895 ±0.074) µg/g and (38.63 ±0.35) µg/g. The associated CVs of 1.07 % and 0.92 % agree well with those of the RFs and the control material.

Table 3. Creatinine Target Estimates for SRM 967b Low and High Levels

SRM 967b Low Level

Set	Box	Aliquot	Injection	Creatinine, µg/g
1	1	1	1	6.808
			2	6.788
			3	6.858
		2	1	6.803
			2	6.839
			3	6.889
	40	1	1	6.922
			2	6.847
			3	6.849
		2	1	6.862
			2	6.855
			3	6.898
2	81	1	1	6.963
			2	6.942
			3	6.932
		2	1	7.031
			2	7.016
			3	7.004

SRM 967b High Level

Set	Box	Aliquot	Injection	Creatinine, µg/g
1	81	1	1	38.33
			2	38.55
			3	38.38
		2	1	38.41
			2	38.70
			3	38.65
2	1	1	1	38.33
			2	38.57
			3	38.13
		2	1	38.57
			2	38.16
			3	38.29
	40	1	1	39.29
			2	39.03
			3	39.17
		2	1	38.83
			2	38.95
			3	39.07

3.4.1. Comparison to Screening Values

The contractor-supplied screening values, determined using an enzymatic method, are expressed as mass concentration in milligrams of creatinine per deciliter of serum, mg/dL. These values can be compared to the above target mass fraction estimates using the relationship.

$$\gamma_{\text{analyte}}\,\frac{\text{mg analyte}}{\text{dL serum}} = \left(w_{\text{analyte}}\,\frac{\text{µg analyte}}{\text{g serum}}\right)\left(\rho\,\frac{\text{g serum}}{\text{mL serum}}\right)\left(\frac{\text{100 mL serum}}{\text{dL serum}}\right)\left(\frac{\text{mg analyte}}{\text{1000 µg analye}}\right) \qquad (5)$$

where ρ is the mass density of the serum.

Assuming the enzymatic values were determined at ambient temperature, an appropriate mass density for normal human serum is 1.02 g/mL. The target value for the low level as determined above is (6.90 ± 0.07)(1.02)(100/1000) = (0.704 ± 0.007) mg/dL; for the high level it is (38.6 ± 0.4)(1.02)(100/1000) = (3.94 ± 0.04) mg/dL. These are in adequate agreement with the 0.64 g/dL and 3.9 g/dL screening values, given the uncertainties of 0.05 g/dL and 0.2 g/dL specified in the Scope of Work (Section 2.1) .

4. Certification Measurements

4.1. Materials

Other than the use of SRM 967a Levels 1 and 2 as control materials rather than SRM 1950, the materials used for certification were the same used to establish the target values as described in Section 3.1.

The number of calibrant and sample preparations required to achieve a desired final expanded uncertainty of ≤2.1 % (similar to SRM 967a Level 1 and Level 2) was informed by results from the Design of Experiment application in the NIST ABACUS Chemical Analysis Package [12], ISO Guide 35 [13] recommendations, and the need to evaluate within-vial variability. The final experimental design required measuring ten vials of each level, with duplicate aliquot prepared from six vials and a single prepared from the remaining four vials.

Random stratified sampling was used to select the ten vials across the batch of each level by first selecting a vial from the first box (1) and last box (81) and then dividing the remaining boxes into 8 relatively even subgroups. One vial within each group of boxes was randomly selected. Sample preparations were divided into four sets, each to be accomplished in one day. Both aliquots of each of the six vials prepared in duplicate were measured in the same set. Table 4 lists the box number from which the vial was taken, the number of aliquots prepared, and the set in which the samples were measured.

Table 4. Selected Vials and Number Replicates

	Low Level			High Level		
Vial	Box	Aliquots	Set	Box	Aliquots	Set
1	1	2	1	1	2	3
2	3	2	2	4	1	1
3	13	2	3	18	1	2
4	22	1	4	27	2	4
5	33	2	2	38	2	1
6	48	1	4	42	1	2
7	58	1	3	55	1	1
8	70	1	3	66	2	4
9	72	2	1	80	2	2
10	81	2	4	81	2	3

4.2. Material Preparation

Reagents, calibrants, the internal standard solution, and samples were prepared as described in Section 3.2.

Table 5 lists the sample preparation order for the SRM 967a and 967b materials in each of the four sets.

Table 5. Sample Preparation Order

	Set 1			Set 2			Set 3			Set 4		
Order	Sample	Vial	Aliquot	Sample	Vial	Aliquot	Sample	Vial	Aliquot	Sample	Vial	Aliquot
1	967a L1	1	1	967a L1 2	1		967a L1	3	1	967a L1	4	1
2	967b Low	1	1	967b Low	5	1	967b Low	3	1	967b Low	6	1
3	967b Low	9	1	967b Low	2	1	967b Low	8	1	967b Low	10	1
4	967b Low	9	2	967b Low	2	2	967b Low	7	1	967b Low	4	1
5	967b Low	1	2	967b Low	5	2	967b Low	3	2	967b Low	10	2
6	967a L1	1	2	967a L1	2	2	967a L1	3	2	967a L1	4	2
7	967a L2	1	1	967a L2	2	1	967a L2	3	1	967a L2	4	1
8	967b High	2	1	967b High	6	1	967b High	10	1	967b High	4	1
9	967b High	5	1	967b High	9	1	967b High	1	1	967b High	8	1
10	967b High	7	1	967b High	3	1	967b High	1	2	967b High	8	2
11	967b High	5	2	967b High	9	2	967b High	10	2	967b High	4	2
12	967a L2	2	2	967a L2 2	2		967a L2	3	2	967a L2	4	2

4.3. Instrumental Method

With the exception that 1) each sample was injected twice rather than three times and 2) the dwell time was 294 ms rather than 195 ms, the equipment, ID-LC-MS method, and preliminary data analysis used were as described in Section 3.3. Section 7 describes the complete statistical evaluation of the SRM 967b measurement results.

Table 6 lists the analysis order for all materials in each of the four sets. Representative chromatograms for the calibrants, SRM 967a Levels 1 and 2, and SRM 967b Low and High Levels are displayed in Fig. 6.

Table 6. Blank, Calibrant, and Sample Analysis Order

	Set 1				Set 2				Set 3				Set 4			
Order	Sample	Vial	Aliquot	Injection	Sample	Vial	Aliquot	Injection	Sample	Vial	Aliquot	Injection	Sample	Vial	Aliquot	Injection
1	Water				Water				Water				Water			
2	Water				Water				Water				Water			
3	Water				Water				Water				Water			
4	Calibrant 1			1	Calibrant 1			1	Calibrant 1			1	Calibrant 1			1
5	Calibrant 2			1	Calibrant 2			1	Calibrant 2			1	Calibrant 2			1
6	Calibrant 3			1	Calibrant 3			1	Calibrant 3			1	Calibrant 3			1
7	Calibrant 4			1	Calibrant 4			1	Calibrant 4			1	Calibrant 4			1
8	Water				Water				Water				Water			
9	967a L1	1	1	1	967b High	6	1	1	967b High	10	1	1	967a L2	4	2	1
10	967b Low	1	1	1	967a L1	2	2	1	967a L1	3	1	1	967a L1	4	1	1
11	967b High	7	1	1	967b High	9	1	1	967b Low	8	1	1	967b High	4	1	1
12	967b Low	9	1	1	967b Low	2	1	1	967a L2	3	2	1	967b Low	10	1	1
13	967a L2	1	1	1	967a L2	2	2	1	967b High	1	1	1	967b High	8	1	1
14	967a L1	1	2	1	967b Low	2	2	1	967b Low	7	1	1	967b Low	10	2	1
15	967b Low	1	2	1	967b Low	5	1	1	967b High	10	2	1	967a L2	4	1	1
16	967b High	2	1	1	967a L2	2	1	1	967b Low	3	1	1	967b High	8	2	1
17	967b High	5	1	1	967b High	3	1	1	967b Low	3	2	1	967b Low	4	1	1
18	967b Low	9	2	1	967b Low	5	2	1	967a L1	3	2	1	967a L1	4	2	1
19	967b High	5	2	1	967b High	9	2	1	967a L2	3	1	1	967b Low	6	1	1
20	967a L2	1	2	1	967a L1	2	1	1	967b High	1	2	1	967b High	4	2	1
21	Water				Water				Water				Water			
22	Calibrant 2			2	Calibrant 2			2	Calibrant 4			2	Calibrant 4			2
23	Calibrant 3			2	Calibrant 4			2	Calibrant 1			2	Calibrant 2			2
24	Calibrant 1			2	Calibrant 3			2	Calibrant 3			2	Calibrant 1			2
25	Calibrant 4			2	Calibrant 1			2	Calibrant 2			2	Calibrant 3			2
26	Water				Water				Water				Water			
27	967a L2	1	2	2	967a L1	2	1	2	967a L1	3	2	2	967a L2	4	2	2
28	967b High	7	1	2	967b High	6	1	2	967b High	10	1	2	967a L1	4	2	2
29	967b High	5	2	2	967b Low	5	2	2	967b Low	3	1	2	967b Low	10	1	2
30	967b Low	9	1	2	967b Low	2	1	2	967a L2	3	2	2	967b High	4	1	2
31	967a L1	1	2	2	967a L2	2	1	2	967b High	1	2	2	967b Low	4	1	2
32	967b Low	1	2	2	967b High	3	1	2	967b Low	7	1	2	967b High	8	1	2
33	967a L2	1	1	2	967b Low	2	2	2	967a L2	3	1	2	967b Low	6	1	2
34	967b High	2	1	2	967b High	9	1	2	967b Low	3	2	2	967b High	8	2	2
35	967b Low	9	2	2	967b Low	5	1	2	967b High	1	1	2	967b Low	10	2	2
36	967b High	5	1	2	967a L1	2	1	2	967a L1	3	1	2	967a L2	4	1	2
37	967b Low	1	1	2	967b High	9	2	2	967b Low	8	1	2	967a L1	4	1	2
38	967a L1	1	1	2	967a L2	2	2	2	967b High	10	2	2	967b High	4	2	2
39	Water				Water				Water				Water			

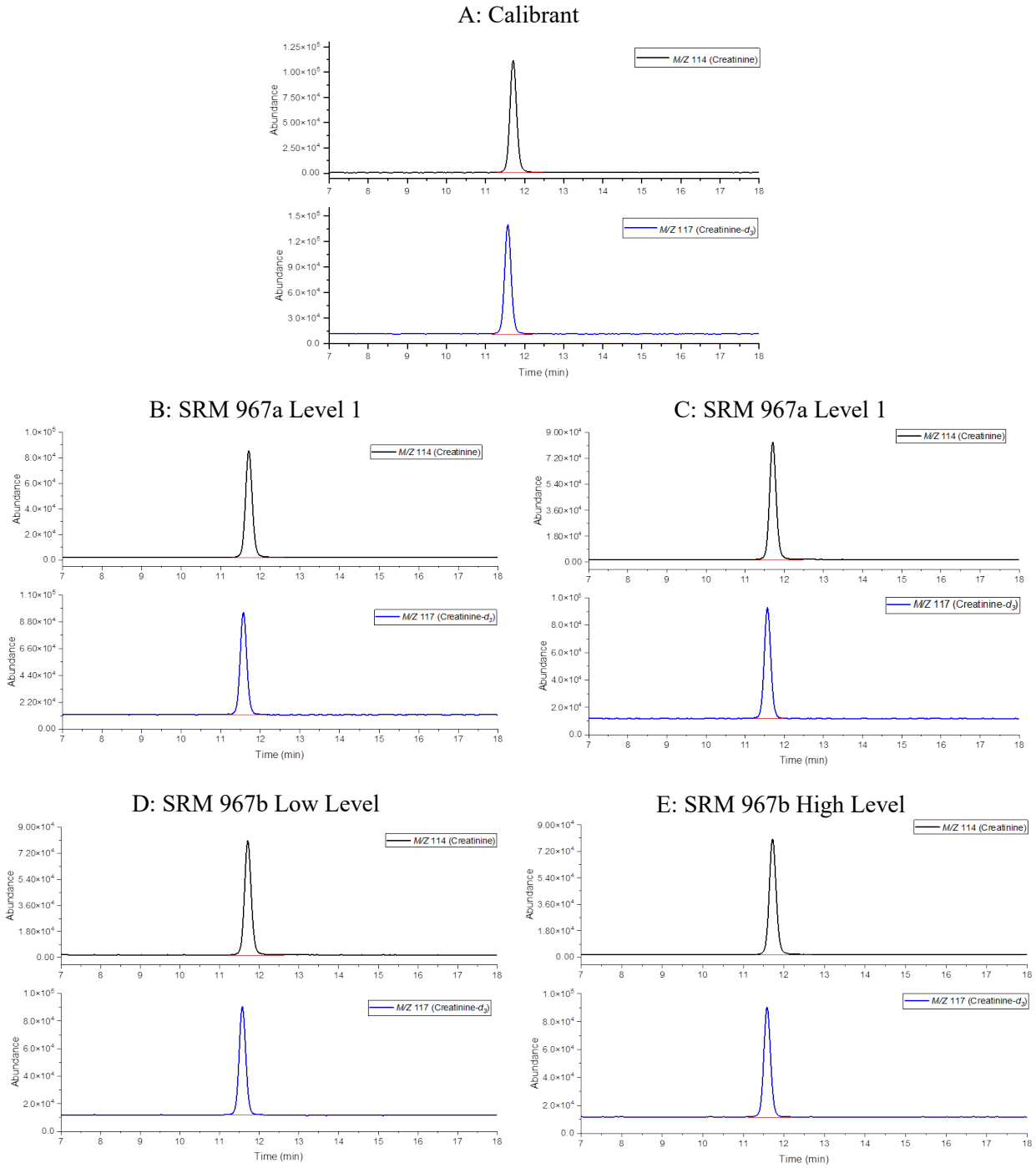

Fig. 6. Representative Value Assignment SIM Chromatograms.

Each panel displays SIM chromatograms at *m*/*z* 114 (upper sub-panel) and *m*/*z* 117 (lower sub panel) of one injection: A) Calibrant, B) SRM 967a Level 1, C) SRM 967a Level 2, D) SRM 967b Low Level sample, and E) SRM 967b High Level sample.

4.4. Results

4.4.1. Stock and Calibrants

Table 7 details the preparation of the stock solutions used to prepare the four calibrants used in all four of the measurement sets. Creatinine calibration stocks 1 to 3 were prepared from one vial of SRM 914b, stock 4 was prepared from a different vial.

Table 7. Creatinine and Creatinine-d_3 Stock Solutions

	Analyte [a]							
	Mass Water, g		Mass, mg		Purity, g/g		Mass Fraction, µg/g	
Stock Solution	m_{water}	$u(m_{water})$ [b]	$m_{analyte}$	$u(m_{analyte})$ [b]	$p_{analyte}$	$u(p_{analyte})$ [c]	$w_{analyte}$	$u(w_{analyte})$
Calibrant Stock 1	79.9493	0.00006	1.2446	0.00006	0.999	0.0005	15.5516	0.0078
Calibrant Stock 2	80.1324	0.00006	1.2567	0.00006	0.999	0.0005	15.6669	0.0079
Calibrant Stock 3	80.0162	0.00006	1.0305	0.00006	0.999	0.0005	12.8656	0.0065
Calibrant Stock 4	78.9265	0.00006	1.0384	0.00006	0.999	0.0005	13.1432	0.0066
IS Stock	80.0154	0.00006	1.1001	0.00006	1	0	13.7484	0.0007

a The "analyte" for the calibrant stock solutions is creatinine sourced from SRM 914b.
The "analyte" for the internal standard (IS) stock solution is commercially obtained creatinine-d_3.

b Mass measurements are assumed to be stable within ±1 least-significant reported digit (lsrd), suggesting a standard uncertainty of $1/\sqrt{3} \approx 0.6$ lsrd.

c SRM 914b has certified purity of (0.999 ± 0.001) g/g, where ± 0.001 is a symmetric 95 % level of uncertainty. The standard uncertainty is one-half of this value: 0.0005 g/g.
The creatinine-d_3 internal standard is nominally "pure".

Table 8 details the mass measurements and mass fraction values used to estimate the $m_{IS}/m_{analyte}$ parameter of Eq. 1. Table 9 details the peak area measurements used to estimate the $A_{analyte}/A_{IS}$ parameter of Eq. 1. Table 10 lists the RFs calculated for each of the four calibrants in each of the four measurement sets.

Table 8. Masses Used to Estimate Control Solution Response Factors [a]

Set	Cal [b]	Internal Standard (Creatinine-d_3) $m_{stockIS}$, g	$w_{stockIS}$, µg/g [c]	Calibrant (Creatinine) $m_{stockAnalyte}$, g	$w_{stockAnalyte}$, µg/g [c]	$m_{IS}/m_{analyte}$
1	1	0.17650 ±0.000006	13.7484 ±0.0005	0.12072 ±0.000006	15.5516 ±0.0078	1.2925 ±0.0005
	2	0.17502 ±0.000006	13.7484 ±0.0005	0.13516 ±0.000006	15.6669 ±0.0078	1.1363 ±0.0005
	3	0.17469 ±0.000006	13.7484 ±0.0005	0.18531 ±0.000006	12.8656 ±0.0064	1.0074 ±0.0005
	4	0.17468 ±0.000006	13.7484 ±0.0005	0.21948 ±0.000006	13.1432 ±0.0066	0.8325 ±0.0005
2	1	0.17274 ±0.000006	13.7484 ±0.0005	0.12160 ±0.000006	15.5516 ±0.0078	1.2558 ±0.0005
	2	0.17543 ±0.000006	13.7484 ±0.0005	0.13635 ±0.000006	15.6669 ±0.0078	1.1291 ±0.0005
	3	0.17488 ±0.000006	13.7484 ±0.0005	0.18549 ±0.000006	12.8656 ±0.0064	1.0075 ±0.0005
	4	0.17514 ±0.000006	13.7484 ±0.0005	0.22082 ±0.000006	13.1432 ±0.0066	0.8297 ±0.0005
3	1	0.17318 ±0.000006	13.7484 ±0.0005	0.12264 ±0.000006	15.5516 ±0.0078	1.2484 ±0.0005
	2	0.17487 ±0.000006	13.7484 ±0.0005	0.13574 ±0.000006	15.6669 ±0.0078	1.1305 ±0.0005
	3	0.17501 ±0.000006	13.7484 ±0.0005	0.18580 ±0.000006	12.8656 ±0.0064	1.0066 ±0.0005
	4	0.17496 ±0.000006	13.7484 ±0.0005	0.21919 ±0.000006	13.1432 ±0.0066	0.8350 ±0.0005
4	1	0.17349 ±0.000006	13.7484 ±0.0005	0.12186 ±0.000006	15.5516 ±0.0078	1.2586 ±0.0005
	2	0.17356 ±0.000006	13.7484 ±0.0005	0.13589 ±0.000006	15.6669 ±0.0078	1.1208 ±0.0005
	3	0.17375 ±0.000006	13.7484 ±0.0005	0.18463 ±0.000006	12.8656 ±0.0064	1.0056 ±0.0005
	4	0.17481 ±0.000006	13.7484 ±0.0005	0.21862 ±0.000006	13.1432 ±0.0066	0.8365 ±0.0005

a Values expressed as value ±standard uncertainty. Mass measurements are assumed to be stable within ±1 least-significant reported digit (lsrd), suggesting a standard uncertainty of $1/\sqrt{3} \approx 0.6$ lsrd.

b Calibrant identifier. The Cal 1 solutions were prepared from Calibration Stock 2, Cal 2 from Calibration Stock 3, Cal 3 from Calibration Stock 1, and Cal 4 from Calibration Stock 4.

c Mass fraction of IS and calibrant solutions are from Table 7.

Table 9. Peak Area Measurements Used to Estimate Control Solution Response Factors

		Peak Area, arbitrary units.				$A_{analyte}/A_{IS}$		
		Injection 1		Injection 2				
Set	Cal	*m/z* 114	*m/z* 117	*m/z* 114	*m/z* 117	Inj 1 [d]	Inj 2 [d]	Mean ±SD
1	1	831895	1074870	1086902	1414280	0.7739	0.7685	0.7712 ±0.0038
	2	920691	1071889	1149541	1328887	0.8589	0.8650	0.8620 ±0.0043
	3	910111	949654	1131358	1178626	0.9584	0.9599	0.9591 ±0.0011
	4	1029123	881305	1283229	1103021	1.1677	1.1634	1.1656 ±0.0031
2	1	408821	519024	630332	799029	0.7877	0.7889	0.7883 ±0.0008
	2	453938	503095	627031	714986	0.9023	0.8770	0.8896 ±0.0179
	3	415866	428335	623008	647828	0.9709	0.9617	0.9663 ±0.0065
	4	477369	395237	686091	569180	1.2078	1.2054	1.2066 ±0.0017
3	1	1292062	1612026	1394330	1762926	0.8015	0.7909	0.7962 ±0.0075
	2	1394254	1566995	1506157	1705477	0.8898	0.8831	0.8864 ±0.0047
	3	1353722	1374461	1451123	1488261	0.9849	0.9750	0.9800 ±0.0070
	4	1500695	1266515	1575214	1332817	1.1849	1.1819	1.1834 ±0.0021
4	1	592250	747471	1078896	1385568	0.7923	0.7787	0.7855 ±0.0097
	2	696440	792656	1096904	1256353	0.8786	0.8731	0.8759 ±0.0039
	3	713905	720796	1131271	1128249	0.9904	1.0027	0.9966 ±0.0087
	4	745063	626194	1161989	990898	1.1898	1.1727	1.1812 ±0.0121

Table 10. Response Factors and Summary Statistics

	Set 1		Set 2		Set 3		Set 4	
	R	*u*(*R*)	*R*	*u*(*R*)	*R*	*u*(*R*)	*R*	*u*(*R*)
	0.9968	0.005	0.9899	0.0011	0.9940	0.0094	0.9886	0.0122
	0.9795	0.0049	1.0044	0.0202	1.0021	0.0053	0.9817	0.0044
	0.9662	0.0011	0.9735	0.0066	0.9864	0.007	1.0022	0.0087
	0.9704	0.0026	1.0011	0.0014	0.9881	0.0018	0.988	0.0102
Mean	0.9782		0.9922		0.9927		0.9901	
Standard Deviation	0.0136		0.0139		0.0071		0.0086	
Pooled uncertainty		0.0038		0.0107		0.0065		0.0093
Standard uncertainty	0.0141		0.0176		0.0096		0.0127	
Standard uncertainty of the Mean	0.0071		0.0088		0.0048		0.0063	
% Relative standard uncertainty	1.44		1.77		0.97		1.28	

The mean within-set RF values for each set are close to 1 (0.978, 0.992, 0.993 and 0.990). The relative standard uncertainties of the RFs (analogous to CV) are relatively small and consistent (1.44, 1.77, 0.97, 1.28) indicating that the preparation of calibrants was consistent, calibrants were stable, and that the peak area ratios were repeatable and reproducible over the experimental period.

4.4.2. SRM 967a Level 1 and Level 2

SRM 967a is certified for creatinine at a mass concentration of (0.847 ±0.018) mg/dL for Level 1 and (3.877 ±0.082) mg/dL for Level 2. Using the density value of 1.02291 g/mL provided on the Certificate of Analysis [4] and Eq. 5, the creatinine mass fractions for the two Levels are (8.28 ±0.18) µg/g and (37.90 ±0.80) µg/g.

The estimated creatinine mass fractions in the SRM 967a control materials are displayed in Fig. 7 as a function of injection sequence. The measurement process provided accurate estimates of creatinine mass fraction.

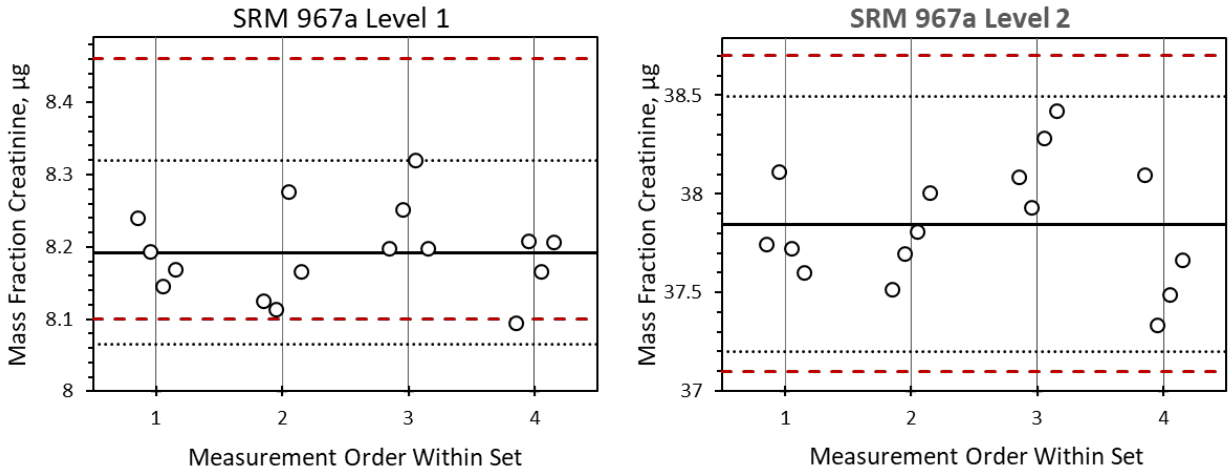

Fig. 7. SRM 967a Level 1 and Level 2 Control Measurements.

Results for SRM 967a Level 1 (left) and Level 2 (right) controls plotted as functions of measurement order within each set. The open circles represent the measurement results for each injection. The solid horizontal lines represent the mean of all results for a given material; the horizontal dotted lines bound the approximate 95 % level of confidence interval about that mean. The horizontal dashed lines represent the 95 % level of confidence certified intervals for the SRM 967a materials converted from mass concentration to mass fraction. The thin vertical lines indicate measurement set.

The mean ± SD creatinine value for control SRM 967a Level 1 was (8.192 µg/g ±0.060 µg/g) and (37.846 µg/g ±0.30 µg/g) for SRM 967a Level 2. These results are within the certified uncertainties. The 0.73 % and 0.80 % CVs for the two materials are small and consistent. The measurement process provided accurate estimates of creatinine mass fraction.

While the results for both control materials in set 3 are on average slightly higher than in the other sets, there is no systematic trend related to measurement sequence. The creatinine measurement process was in adequate control throughout the measurement campaign.

The bias in the set 3 results is more directly visualized in Fig. 8. Since a different pair of vials was evaluated in each set, the bias may arise from: 1) the mass fraction of creatinine in both the Level 1 and Level 2 vials used being slightly high and/or 2) the RF for set 3 being slightly low.

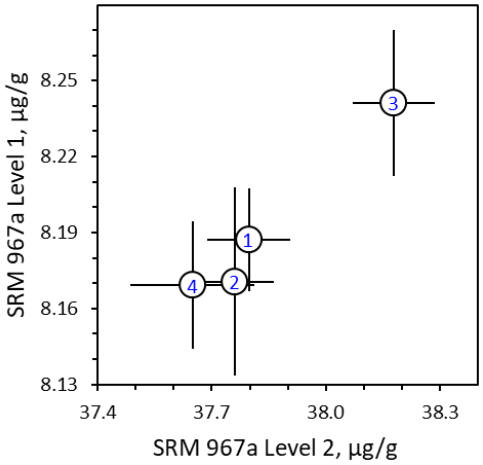

Fig. 8. Within-Set Mean Values for SRM 967a Level 1 as a Function of Level 2.

Each open circle represents the mean result of the four evaluations of SRM 967a Level 1 in one of the preparation sets plotted as a function of the mean result for the four evaluations of SRM 967a Level 2 in the same set. The circles are labeled with the set index. The error crosses denote standard uncertainties of the mean.

Table 11 details the peak areas, mass measurements, and parameters used to estimate the mass fraction of creatinine in the SRM 967a Level 1 control material. Table 12 details the peak areas, mass measurements, and parameters used to estimate the mass fraction of creatinine in the SRM 967a Level 2 control material.

Table 11. P Parameters for Estimating Mass Fraction Creatinine in SRM 967a Level 1. [a]

Set	Vial	Alq [b]	Inj [c]	$A_{analyte}/A_{IS}$			m_{IS} µg [d]		R [e]	m_{sample}, g	$w_{analyte}$, µg/g [f]
				m/z 114	*m/z* 117	A_{114}/A_{117}	$m_{stockIS}$, g	$w_{stockIS}$, µgg			
1	1	1	1	687941	688296	0.99948	0.17424 ±0.000006	13.7484 ±0.0005	0.9782 ±0.0071	0.29704 ±0.000006	8.240 ±0.060
			2	936049	944746	0.99079	0.17424 ±0.000006	13.7484 ±0.0005	0.9782 ±0.0071	0.29704 ±0.000006	8.168 ±0.059
		2	1	742517	756979	0.98090	0.17528 ±0.000006	13.7484 ±0.0005	0.9782 ±0.0071	0.29489 ±0.000006	8.194 ±0.059
			2	872230	894522	0.97508	0.17528 ±0.000006	13.7484 ±0.0005	0.9782 ±0.0071	0.29489 ±0.000006	8.146 ±0.059
2	2	1	1	387235	387775	0.99861	0.17535 ±0.000006	13.7484 ±0.0005	0.9922 ±0.0088	0.29902 ±0.000006	8.114 ±0.072
			2	468953	460393	1.01859	0.17535 ±0.000006	13.7484 ±0.0005	0.9922 ±0.0088	0.29902 ±0.000006	8.277 ±0.073
		2	1	305184	304356	1.00272	0.17509 ±0.000006	13.7484 ±0.0005	0.9922 ±0.0088	0.29789 ±0.000006	8.167 ±0.072
			2	608823	610291	0.99759	0.17509 ±0.000006	13.7484 ±0.0005	0.9922 ±0.0088	0.29789 ±0.000006	8.125 ±0.072
3	3	1	1	971658	969039	1.00270	0.17511 ±0.000006	13.7484 ±0.0005	0.9926 ±0.0048	0.29665 ±0.000006	8.197 ±0.040
			2	1068645	1065667	1.00279	0.17511 ±0.000006	13.7484 ±0.0005	0.9926 ±0.0048	0.29665 ±0.000006	8.198 ±0.040
		2	1	1032480	1014501	1.01772	0.17478 ±0.000006	13.7484 ±0.0005	0.9926 ±0.0048	0.29859 ±0.000006	8.250 ±0.040
			2	1072623	1045281	1.02616	0.17478 ±0.000006	13.7484 ±0.0005	0.9926 ±0.0048	0.29859 ±0.000006	8.319 ±0.040
4	4	1	1	529076	532161	0.99420	0.17418 ±0.000006	13.7484 ±0.0005	0.9901 ±0.0063	0.29704 ±0.000006	8.095 ±0.052
			2	967009	959449	1.00788	0.17418 ±0.000006	13.7484 ±0.0005	0.9901 ±0.0063	0.29704 ±0.000006	8.207 ±0.052
		2	1	679538	682953	0.99500	0.17624 ±0.000006	13.7484 ±0.0005	0.9901 ±0.0063	0.29663 ±0.000006	8.209 ±0.052
			2	891755	900961	0.98978	0.17624 ±0.000006	13.7484 ±0.0005	0.9901 ±0.0063	0.29663 ±0.000006	8.166 ±0.052

a Values are expressed as value ± standard uncertainty.
b Aliquot of Vial
c Injection of aliquot
d m_{IS} is calculated as the product of $m_{stockIS}$ and $w_{stockIS}$. The value for $w_{stockIS}$ is from Table 7.
e Response factor is from Table 10.
f The standard uncertainty does not include any contribution from the determination of the peak areas.

Table 12. Parameters for Estimating Mass Fraction Creatinine in SRM 967a Level 2. [a]

Set	Vial	Alq [b]	Inj [c]	$A_{analyte}/A_{IS}$			m_{IS} µg [d]		R [e]	m_{sample}, g	$w_{analyte}$, µg/g [f]
				m/z 114	*m/z* 117	A_{114}/A_{117}	$m_{stockIS}$, g	$w_{stockIS}$, µgg			
1	1	1	1	741987	734309	1.01046	0.17555 ±0.000006	13.7484 ±0.0005	0.9782 ±0.0071	0.06605 ±0.000006	37.75 ±0.27
			2	916754	910703	1.00664	0.17555 ±0.000006	13.7484 ±0.0005	0.9782 ±0.0071	0.06605 ±0.000006	37.60 ±0.27
		2	1	782356	773758	1.01111	0.17492 ±0.000006	13.7484 ±0.0005	0.9782 ±0.0071	0.06522 ±0.000006	38.11 ±0.28
			2	839906	839232	1.00080	0.17492 ±0.000006	13.7484 ±0.0005	0.9782 ±0.0071	0.06522 ±0.000006	37.73 ±0.27
2	2	1	1	351516	346126	1.01557	0.17493 ±0.000006	13.7484 ±0.0005	0.9922 ±0.0088	0.06530 ±0.000006	37.70 ±0.33
			2	538894	529001	1.01870	0.17493 ±0.000006	13.7484 ±0.0005	0.9922 ±0.0088	0.06530 ±0.000006	37.81 ±0.34
		2	1	338046	332730	1.01598	0.17497 ±0.000006	13.7484 ±0.0005	0.9922 ±0.0088	0.06565 ±0.000006	37.52 ±0.33
			2	675322	656112	1.02928	0.17497 ±0.000006	13.7484 ±0.0005	0.9922 ±0.0088	0.06565 ±0.000006	38.01 ±0.33
3	3	1	1	1004025	990729	1.01342	0.17486 ±0.000006	13.7484 ±0.0005	0.9926 ±0.0048	0.06470 ±0.000006	37.93 ±0.18
			2	1062399	1035050	1.02642	0.17486 ±0.000006	13.7484 ±0.0005	0.9926 ±0.0048	0.06470 ±0.000006	38.42 ±0.19
		2	1	1006644	982271	1.02481	0.17484 ±0.000006	13.7484 ±0.0005	0.9926 ±0.0048	0.06516 ±0.000006	38.08 ±0.18
			2	1080983	1049386	1.03011	0.17484 ±0.000006	13.7484 ±0.0005	0.9926 ±0.0048	0.06516 ±0.000006	38.28 ±0.19
4	4	1	1	647430	645413	1.00313	0.17467 ±0.000006	13.7484 ±0.0005	0.9901 ±0.0063	0.06516 ±0.000006	37.34 ±0.24
			2	973089	961578	1.01197	0.17467 ±0.000006	13.7484 ±0.0005	0.9901 ±0.0063	0.06516 ±0.000006	37.67 ±0.24
		2	1	518266	499988	1.03656	0.17448 ±0.000006	13.7484 ±0.0005	0.9901 ±0.0063	0.06592 ±0.000006	38.10 ±0.24
			2	899471	881780	1.02006	0.17448 ±0.000006	13.7484 ±0.0005	0.9901 ±0.0063	0.06592 ±0.000006	37.49 ±0.24

a Values are expressed as value ±standard uncertainty.
b Aliquot of Vial
c Injection of aliquot
d m_{IS} is calculated as the product of $m_{stockIS}$ and $w_{stockIS}$. The value for $w_{stockIS}$ is from Table 7.
e Response factor is from Table 10.
f The standard uncertainty does not include any contribution from the determination of the peak areas.

4.4.3. SRM 967b Low Level and High Level

The estimated creatinine mass fractions for the 32 analyses (four sets of eight evaluations per set) of the SRM 967b Low Level and High Level materials are displayed in Fig. 9. There is no evidence for systematic injection-order trends in either material.

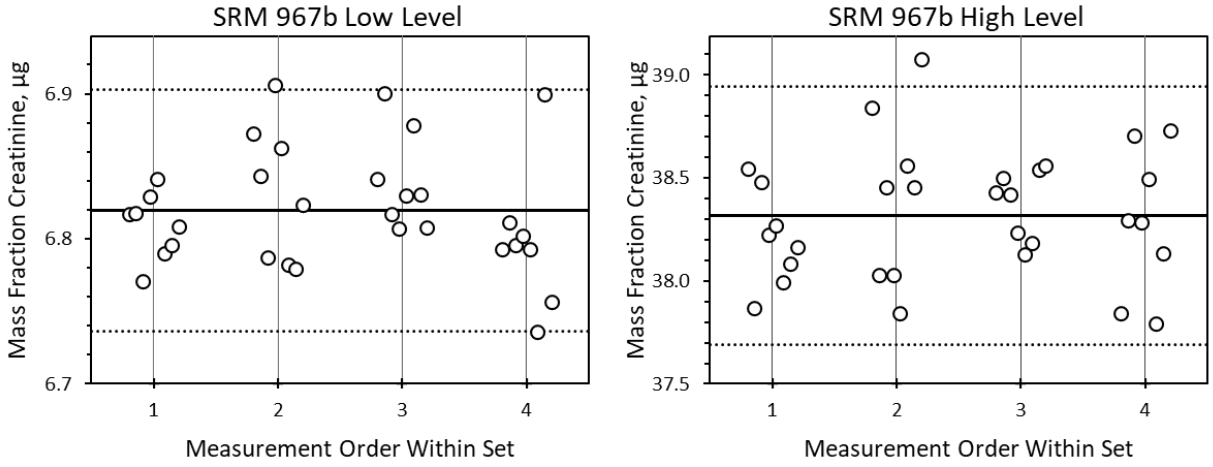

Fig. 9. SRM 967b Measurement Results As a Function of Injection Order.

SRM 967b Low Level (left) and High Level (right) results plotted as functions of measurement order within each set. The open circles represent the measurement results for each injection. The solid horizontal lines represent the mean of all results for a given material; the horizontal dotted lines bound the approximate 95 % level of confidence interval about that mean. The thin vertical lines indicate measurement set.

The mean of the two injections of each prepared sample aliquot is displayed in Fig. 10 as a function of sample preparation order within each set. There is no evidence for systematic injection-order trends in either material.

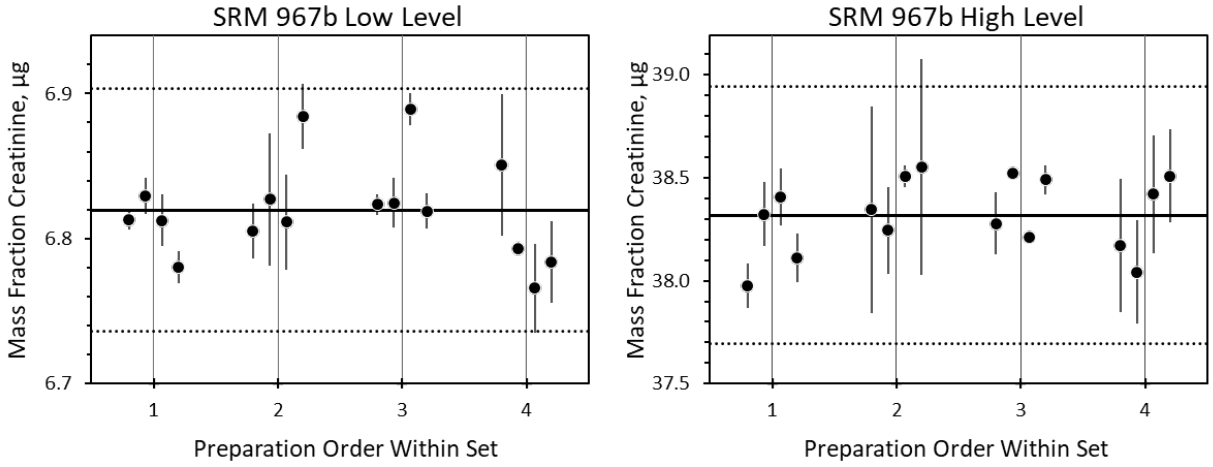

Fig. 10. SRM 967b Measurement Results as a Function of Sample Preparation Order.

SRM 967b Low Level (left) and High Level (right) results plotted as functions of sample preparation order within each set. The solid circles represent the mean measurement results for each aliquot. The solid horizontal lines represent the mean of all results for a given material; the horizontal dotted lines bound the approximate 95 % level of confidence interval about that mean. The thin vertical lines indicate measurement set.

The mean of all injections for each vial are displayed in Fig. 11 as a function of the box from which the vial was taken, which is proportional to the contractor's vial packaging sequence. There is no evidence for a systematic packaging-order trend in either material.

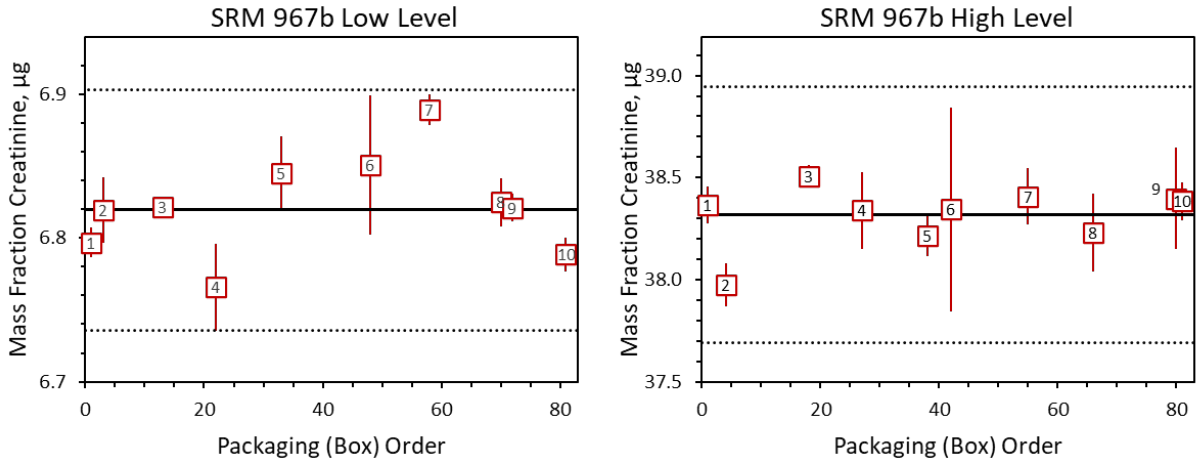

Fig. 11. SRM 967b Measurement Results as a Function of Packaging Order.

Results for SRM 967b Low Level (left) and High Level (right) vials plotted in as a function of box number. The open squares represent the mean of the measurement results for each vial; the squares are labeled with the vial number. The error bars represent one standard uncertainty of the mean. The solid horizontal lines represent the mean of all results for a given material; the horizontal dotted lines bound the approximate 95 % level of confidence interval about that mean.

Based on the mean estimates for the ten vials of each of the SRM 976b materials, the creatinine mass fraction of the Low Level is (6.822 ± 0.041) µg/g and that of the High Level is (38.32 ± 0.26) µg/g. The CVs of the two materials are 0.61 % and 0.67 % are consistent with the repeatability of the set-to-set sample preparation for the SRM 967a control materials.

The slight bias in the set 3 results for the SRM 967a controls is less apparent in the SRM 967b samples as displayed in Fig. 12. This suggests that the between-set differences likely arise from a combination of small intrinsic between-vial compositional differences and between-set calibrant preparations.

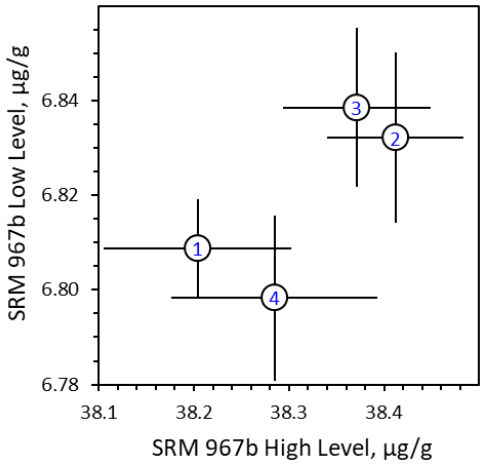

Fig. 12. Within-Set Mean Values for SRM 967a Level 1 as a Function of Level 2.

Each open circle represents the mean result of the four evaluations of SRM 967b Low Level in one preparation set plotted as a function of the mean result for the four evaluations of SRM 967b High Level in the same set. The circles are labeled with the set index. The error crosses denote standard uncertainties of the mean.

Table 13 details the peak areas, mass measurements, and parameters used to estimate the mass fraction of creatinine in the SRM 967b Low Level material. Table 14 details the peak areas, mass measurements, and parameters used to estimate the mass fraction of creatinine in the SRM 967b High Level material.

Table 13. Parameters for Estimating Mass Fraction Creatinine in SRM 967b Low Level. [a]

				$A_{analyte}/A_{IS}$			m_{IS} µg [d]					
Set	Vial	Alq [b]	Inj [c]	*m/z* 114	*m/z* 117	A_{114}/A_{117}	$m_{stockIS}$, g	$w_{stockIS}$, µgg	R [e]	m_{sample}, g	$w_{analyte}$, µg/g [f]
1	1	1	1	767786	764892	1.00378	0.17543 ±0.000006	13.7484 ±0.0005	0.9782 ±0.0071	0.36306 ±0.000006	6.817 ±0.049
			2	1022907	1020282	1.00257	0.17543 ±0.000006	13.7484 ±0.0005	0.9782 ±0.0071	0.36306 ±0.000006	6.809 ±0.049
		2	1	744118	743276	1.00113	0.17519 ±0.000006	13.7484 ±0.0005	0.9782 ±0.0071	0.36408 ±0.000006	6.771 ±0.049
			2	879777	876265	1.00401	0.17519 ±0.000006	13.7484 ±0.0005	0.9782 ±0.0071	0.36408 ±0.000006	6.790 ±0.049
	9	1	1	728167	720721	1.01033	0.17505 ±0.000006	13.7484 ±0.0005	0.9782 ±0.0071	0.36458 ±0.000006	6.818 ±0.049
			2	876999	865120	1.01373	0.17505 ±0.000006	13.7484 ±0.0005	0.9782 ±0.0071	0.36458 ±0.000006	6.841 ±0.050
		2	1	794289	792661	1.00205	0.17525 ±0.000006	13.7484 ±0.0005	0.9782 ±0.0071	0.36140 ±0.000006	6.829 ±0.050
			2	923229	925923	0.99709	0.17525 ±0.000006	13.7484 ±0.0005	0.9782 ±0.0071	0.36140 ±0.000006	6.796 ±0.049
2	2	1	1	329169	318266	1.03426	0.17500 ±0.000006	13.7484 ±0.0005	0.9922 ±0.0088	0.36493 ±0.000006	6.872 ±0.061
			2	529973	519244	1.02066	0.17500 ±0.000006	13.7484 ±0.0005	0.9922 ±0.0088	0.36493 ±0.000006	6.782 ±0.060
		2	1	352087	342533	1.02789	0.17498 ±0.000006	13.7484 ±0.0005	0.9922 ±0.0088	0.36417 ±0.000006	6.844 ±0.061
			2	582052	571614	1.01826	0.17498 ±0.000006	13.7484 ±0.0005	0.9922 ±0.0088	0.36417 ±0.000006	6.779 ±0.060
	5	1	1	354689	347405	1.02097	0.17507 ±0.000006	13.7484 ±0.0005	0.9922 ±0.0088	0.36491 ±0.000006	6.787 ±0.060
			2	621136	605175	1.02637	0.17507 ±0.000006	13.7484 ±0.0005	0.9922 ±0.0088	0.36491 ±0.000006	6.823 ±0.061
		2	1	379106	367755	1.03087	0.17519 ±0.000006	13.7484 ±0.0005	0.9922 ±0.0088	0.36236 ±0.000006	6.906 ±0.061
			2	508987	496848	1.02443	0.17519 ±0.000006	13.7484 ±0.0005	0.9922 ±0.0088	0.36236 ±0.000006	6.863 ±0.061
3	3	1	1	1026377	1010344	1.01587	0.17513 ±0.000006	13.7484 ±0.0005	0.9926 0.0048	0.36147 ±0.000006	6.816 ±0.033
			2	1078770	1059931	1.01777	0.17513 ±0.000006	13.7484 ±0.0005	0.9926 0.0048	0.36147 ±0.000006	6.829 ±0.033
		2	1	935937	928721	1.00777	0.17525 ±0.000006	13.7484 ±0.0005	0.9926 0.0048	0.35936 ±0.000006	6.806 ±0.033
			2	1002023	990855	1.01127	0.17525 ±0.000006	13.7484 ±0.0005	0.9926 0.0048	0.35936 ±0.000006	6.830 ±0.033
	7	1	1	989837	969353	1.02113	0.17517 ±0.000006	13.7484 ±0.0005	0.9926 0.0048	0.35906 ±0.000006	6.899 ±0.033
			2	1062351	1043600	1.01797	0.17517 ±0.000006	13.7484 ±0.0005	0.9926 0.0048	0.35906 ±0.000006	6.878 ±0.033
	8	1	1	968778	959132	1.01006	0.17492 ±0.000006	13.7484 ±0.0005	0.9926 0.0048	0.35769 ±0.000006	6.841 ±0.033
			2	1063153	1057771	1.00509	0.17492 ±0.000006	13.7484 ±0.0005	0.9926 0.0048	0.35769 ±0.000006	6.807 ±0.033
4	4	1	1	646994	638947	1.01259	0.17457 ±0.000006	13.7484 ±0.0005	0.9901 0.0063	0.36119 ±0.000006	6.796 ±0.043
			2	927675	924320	1.00363	0.17457 ±0.000006	13.7484 ±0.0005	0.9901 0.0063	0.36119 ±0.000006	6.736 ±0.043
	6	1	1	690992	682174	1.01293	0.17436 ±0.000006	13.7484 ±0.0005	0.9901 0.0063	0.36055 ±0.000006	6.802 ±0.043
			2	949831	924462	1.02744	0.17436 ±0.000006	13.7484 ±0.0005	0.9901 0.0063	0.36055 ±0.000006	6.899 ±0.044
	10	1	1	567927	557680	1.01837	0.17438 ±0.000006	13.7484 ±0.0005	0.9901 0.0063	0.36301 ±0.000006	6.793 ±0.043
			2	896449	880265	1.01839	0.17438 ±0.000006	13.7484 ±0.0005	0.9901 0.0063	0.36301 ±0.000006	6.793 ±0.043
		2	1	572706	586037	0.97725	0.17449 ±0.000006	13.7484 ±0.0005	0.9901 0.0063	0.34763 ±0.000006	6.811 ±0.043
			2	896368	924715	0.96935	0.17449 ±0.000006	13.7484 ±0.0005	0.9901 0.0063	0.34763 ±0.000006	6.756 ±0.043

a) Values are expressed as value ±standard uncertainty
b) Aliquot of Vial
c) Injection of aliquot
d) m_{IS} is calculated as the product of $m_{stockIS}$ and $w_{stockIS}$. The value for $w_{stockIS}$ is from Table 7
e) Response factor is from Table 10
f) The standard uncertainty does not include any contribution from the determination of the peak areas

Table 14. Parameters for Estimating Mass Fraction Creatinine in SRM 967b High Level. [a]

				$A_{analyte}/A_{IS}$			m_{IS} µg [d]					
Set	Vial	Alq [b]	Inj [c]	*m/z* 114	*m/z* 117	A_{114}/A_{117}	$m_{stockIS}$, g	$w_{stockIS}$, µgg	R [e]	m_{sample}, g	$w_{analyte}$, µg/g [f]	
1	2	1	1	751743	780982	0.96256	0.17565 ±0.000006	13.7484 ±0.0005	0.9782 ±0.0071	0.06275 ±0.000006	37.87	±0.27
			2	907509	937551	0.96796	0.17565 ±0.000006	13.7484 ±0.0005	0.9782 ±0.0071	0.06275 ±0.000006	38.08	±0.28
	5	1	1	786376	783620	1.00352	0.17524 ±0.000006	13.7484 ±0.0005	0.9782 ±0.0071	0.06423 ±0.000006	38.48	±0.28
			2	953875	958362	0.99532	0.17524 ±0.000006	13.7484 ±0.0005	0.9782 ±0.0071	0.06423 ±0.000006	38.17	±0.28
		2	1	762273	768317	0.99213	0.17523 ±0.000006	13.7484 ±0.0005	0.9782 ±0.0071	0.06392 ±0.000006	38.23	±0.28
			2	845950	857867	0.98611	0.17523 ±0.000006	13.7484 ±0.0005	0.9782 ±0.0071	0.06392 ±0.000006	37.99	±0.28
	7	1	1	705907	706607	0.99901	0.17542 ±0.000006	13.7484 ±0.0005	0.9782 ±0.0071	0.06390 ±0.000006	38.55	±0.28
			2	856958	864016	0.99183	0.17542 ±0.000006	13.7484 ±0.0005	0.9782 ±0.0071	0.06390 ±0.000006	38.27	±0.28
2	3	1	1	365514	357436	1.02260	0.17488 ±0.000006	13.7484 ±0.0005	0.9922 ±0.0088	0.06444 ±0.000006	38.45	±0.34
			2	560002	546104	1.02545	0.17488 ±0.000006	13.7484 ±0.0005	0.9922 ±0.0088	0.06444 ±0.000006	38.56	±0.34
	6	1	1	304282	293306	1.03742	0.17529 ±0.000006	13.7484 ±0.0005	0.9922 ±0.0088	0.06487 ±0.000006	38.84	±0.34
			2	490548	485333	1.01075	0.17529 ±0.000006	13.7484 ±0.0005	0.9922 ±0.0088	0.06487 ±0.000006	37.84	±0.34
		1	1	302203	301865	1.00112	0.17516 ±0.000006	13.7484 ±0.0005	0.9922 ±0.0088	0.06389 ±0.000006	38.03	±0.34
	9		2	562097	555263	1.01231	0.17516 ±0.000006	13.7484 ±0.0005	0.9922 ±0.0088	0.06389 ±0.000006	38.46	±0.34
		2	1	381827	380313	1.00398	0.17516 ±0.000006	13.7484 ±0.0005	0.9922 ±0.0088	0.06408 ±0.000006	38.03	±0.34
			2	641259	621578	1.03166	0.17516 ±0.000006	13.7484 ±0.0005	0.9922 ±0.0088	0.06408 ±0.000006	39.08	±0.35
3	1	1	1	995315	979612	1.01603	0.17498 ±0.000006	13.7484 ±0.0005	0.9926 0.0048	0.06396 ±0.000006	38.50	±0.19
			2	1079191	1061030	1.01712	0.17498 ±0.000006	13.7484 ±0.0005	0.9926 0.0048	0.06396 ±0.000006	38.54	±0.19
		2	1	1067924	1055831	1.01145	0.17484 ±0.000006	13.7484 ±0.0005	0.9926 0.0048	0.06406 ±0.000006	38.23	±0.18
			2	1116152	1105051	1.01005	0.17484 ±0.000006	13.7484 ±0.0005	0.9926 0.0048	0.06406 ±0.000006	38.18	±0.18
	10	1	1	978267	954293	1.02512	0.17500 ±0.000006	13.7484 ±0.0005	0.9926 0.0048	0.06466 ±0.000006	38.42	±0.19
			2	1053216	1035528	1.01708	0.17500 ±0.000006	13.7484 ±0.0005	0.9926 0.0048	0.06466 ±0.000006	38.12	±0.18
		2	1	1019043	1004578	1.01440	0.17468 ±0.000006	13.7484 ±0.0005	0.9926 0.0048	0.06388 ±0.000006	38.42	±0.19
			2	1102913	1083277	1.01813	0.17468 ±0.000006	13.7484 ±0.0005	0.9926 0.0048	0.06388 ±0.000006	38.56	±0.19
4	4	1	1	560114	547335	1.02335	0.17447 ±0.000006	13.7484 ±0.0005	0.9901 0.0063	0.06551 ±0.000006	37.85	±0.24
			2	920597	884454	1.04086	0.17447 ±0.000006	13.7484 ±0.0005	0.9901 0.0063	0.06551 ±0.000006	38.49	±0.24
		2	1	761564	745545	1.02149	0.17441 ±0.000006	13.7484 ±0.0005	0.9901 0.0063	0.06462 ±0.000006	38.28	±0.24
			2	1031363	997938	1.03349	0.17441 ±0.000006	13.7484 ±0.0005	0.9901 0.0063	0.06462 ±0.000006	38.73	±0.25
	8	1	1	560573	553650	1.01250	0.17464 ±0.000006	13.7484 ±0.0005	0.9901 0.0063	0.06412 ±0.000006	38.29	±0.24
			2	900953	901621	0.99926	0.17464 ±0.000006	13.7484 ±0.0005	0.9901 0.0063	0.06412 ±0.000006	37.79	±0.24
		2	1	640968	656680	0.97607	0.18542 ±0.000006	13.7484 ±0.0005	0.9901 0.0063	0.06493 ±0.000006	38.70	±0.25
			2	943619	981204	0.96170	0.18542 ±0.000006	13.7484 ±0.0005	0.9901 0.0063	0.06493 ±0.000006	38.13	±0.24

a) Values are expressed as value ±standard uncertainty b) Aliquot of Vial c) Injection of aliquot

d) m_{IS} is calculated as the product of $m_{stockIS}$ and $w_{stockIS}$. The value for $w_{stockIS}$ is from Table 7 e) Response factor is from Table 10

f) The standard uncertainty does not include any contribution from the determination of the peak areas

5. Serum Density Determinations

Density values are needed to express mass and amount-of-substance concentrations of creatinine in the SRM 967b materials. Density values at the reference temperature of 23 °C were determined from gravimetric mass measurements using the Lang-Levy pipet method [14] with sample volumes of approximately 500 µL.

5.1. Materials

HPLC grade water for calibration and pipet rinsing was obtained from JT Baker. Ethanol (200 proof) for rinsing pipets was obtained from Warner-Graham.

Three vials each of SRM 967b Low Level (from boxes 11, 29, and 41) and SRM 967b High Level (from boxes 11, 27, and 48) were removed from -80 °C storage and left undisturbed to thaw at room temperature for one and half hours each. The three vials of each level were combined into individual 4 mL glass vials.

5.2. Method

A 500 µL Lang-Levy pipet was calibrated with water that had been equilibrated for at least 24 h at ambient balance room temperature. The temperature of the room was measured using a 2626S Thermo-Hygrometer (Fluke Corporation) at the beginning and end of each set of measurements.

Using a Mettler Toledo XPE205 analytical balance, a metal pipet stand was tared, a clean and dry pipet was carefully placed on the stand, and the mass of the pipet was recorded. The pipet was then filled to the mark with water, wiped with a lint-free cloth, and weighed. To clean and dry the pipet, the pipet was attached to a vacuum trap and several mL of water were pulled through the pipet followed by several mL of ethanol. The pipet remained attached to the vacuum trap and air was pulled through the pipet for 10 minutes to ensure it was completely dry. The water mass determinations were performed in triplicate. The serum mass determinations were performed in triplicate. Between each weighing, the pipet was rinsed with water, then ethanol, and dried for 10 minutes under vacuum.

The balance room temperature remained within (19.5 ± 0.3) °C throughout the measurements.

5.3. Analysis

The contained volume of serum at temperature T is equal to the contained volume of water at that temperature: $V_{\text{serum},T} = V_{\text{water},T}$. The density of serum relative to the density of water at the same temperature is thus given by the mass ratio:

$$\rho_{\text{serum,relative}} = \rho_{\text{serum},T}/\rho_{\text{water},T} = \frac{m_{\text{serum}}}{V_{\text{serum},T}}\Big/\frac{m_{\text{water}}}{V_{\text{water},T}} = m_{\text{serum}}/m_{\text{water}} \quad (6)$$

The absolute serum density at 23 °C is then estimated from the 0.99756 g/mL reference density of water at 23 °C given in [14] and assuming a conservative standard uncertainty for that value of 0.00010 g/mL:

$$\rho_{\text{serum},23°\text{C}} = \left(\rho_{\text{serum,relative}}\right)\left(\rho_{\text{water},23°\text{C}}\right) = \frac{m_{\text{serum}}}{m_{\text{water}}}(0.99756 \pm 0.00010)\ \text{g/mL} \quad (7)$$

Small differences in room temperature between the start of the water measurements, T_{start}, and the end of the serum measurements, T_{end}, can be corrected for using the volumetric expansion formula given in [14] and assuming conservative standard uncertainties for the temperature measurements of 0.01 °C and for the correction coefficient of 0.00001:

$$V_{23} = V_T\left(1 - (0.00021 \pm 0.00001)\big((T \pm 0.01) - 23\big)\right) \quad (8)$$

Since this correction is asserted to be appropriate for both water and serum:

$$V_{\text{water},T_{\text{start}}} = V_{23}/\left(1 - (0.00021 \pm 0.00001)\big((T_{\text{start}} \pm 0.01) - 23\big)\right)$$

$$V_{\text{serum},T_{\text{end}}} = V_{23}/\left(1 - (0.00021 \pm 0.00001)\big((T_{\text{end}} \pm 0.01) - 23\big)\right)$$

Substituting these into Eqs. 6 and 7:

$$\rho_{\text{serum,23°C}} = \left(\frac{m_{\text{serum}}}{m_{\text{water}}}\right)\left(\frac{1-(0.00021\pm0.00001)\big((T_{\text{start}}\pm0.01)-23\big)}{1-(0.00021\pm0.00001)\big((T_{\text{end}}\pm0.01)-23\big)}\right)(0.99756\ \pm 0.00010)\ \text{g/mL} \quad (9)$$

5.3.1. SRM 967b Low Level

SRM 967b Low Level was evaluated first. The balance room temperature was 19.31 °C at the start of measurements and 19.64 °C at the end. Table 15 lists the measured masses, estimated mass differences, and summary statistics.

Table 15. Mass Measurements for the Density Determination of SRM 967b Low Level.

	HPLC Water			SRM 967b Low Level		
	m_{empty}, g	m_{full}, g	m_{water}, g [a]	m_{empty}, g	m_{full}, g	m_{serum}, g [a]
	8.38897	8.89703	0.50806 ±0.00001	8.38899	8.90916	0.52017 ±0.00001
	8.38897	8.89663	0.50766 ±0.00001	8.38902	8.90909	0.52007 ±0.00001
	8.38897	8.89670	0.50773 ±0.00001	8.38903	8.90859	0.51956 ±0.00001
$\bar{x}$ [b]			0.50782			0.51993
s [b]			0.00021			0.00033
$\bar{u}$ [b]			0.00001			0.00001
$u(\bar{x})$ [b]			0.00012			0.00019

a) All direct mass measurements are assumed to be stable within ±0.00001 g, suggesting a standard uncertainty of 0.00001/√3 = 0.000006 g. The combined standard uncertainty of the $m_{\text{full}} - m_{\text{empty}}$ mass difference is then $\sqrt{0.000006^2 + 0.000006^2} = 0.000008 \approx 0.00001$ g.

b) $\bar{x}$: mean; s: standard deviation; $\bar{u}$: pooled standard uncertainty of individual values; $u(\bar{x})$: standard uncertainty of the mean, $u(\bar{x}) = \sqrt{(s^2 + \bar{u}^2)/3}$.

The density of SRM 967b Low Level at the reference 23 °C is therefore:

$$\rho_{23\text{serum}} = \left(\frac{0.51993 \pm 0.00019}{0.50782 \pm 0.00012}\right)\left(\frac{1 - (0.00021 \pm 0.00001)(19.31 \pm 0.01 - 23)}{1 - (0.00021 \pm 0.00001)(19.64 \pm 0.01 - 23)}\right)(0.99756\ \pm 0.00001)$$
$$= (1.02142 \pm 0.00046)\frac{\text{g}}{\text{mL}}.$$

For two degrees of freedom, the Student's t_{95} expansion factor, k_{95}, is 4.3, for an approximate 95 % level of confidence expanded uncertainty interval, $\rho_{23\text{serum}} \pm U_{95}$, of:

$$\rho_{23\text{serum}} \pm U_{95} = \rho_{23\text{serum}} \pm k_{95}u = 1.02142\ \pm (4.3)(0.00046) = (1.02142\ \pm 0.0020)\frac{\text{g}}{\text{mL}}$$

5.3.2. SRM 967b High Level

SRM 967b High Level was then evaluated. The balance room temperature was 19.54 °C at the start of measurements and 19.79 °C at the end. Table 16 lists the measured masses, estimated mass differences, and summary statistics.

Table 16. Mass Measurements for the Density Determination of SRM 967b High Level.

	HPLC Water				SRM 967b High Level			
	m_{empty}, g	m_{full}, g	m_{water}, g [a]		m_{empty}, g	m_{full}, g	m_{serum}, g [a]	
	8.38898	8.89673	0.50775	±0.00001	8.38908	8.91019	0.52111	±0.00001
	8.38904	8.89667	0.50763	±0.00001	8.38908	8.91002	0.52094	±0.00001
	8.38913	8.89672	0.50759	±0.00001	8.3891	8.90998	0.52088	±0.00001
$\bar{x}$ [b]			0.50766				0.52098	
s [b]			0.00008				0.00012	
$\bar{u}$ [b]				0.00001				0.00001
$u(\bar{x})$ [b]			0.00005				0.00007	

a) All direct mass measurements are assumed to be stable within ±0.00001 g, suggesting a standard uncertainty of 0.00001/√3 = 0.000006 g. The combined standard uncertainty of the $m_{full} - m_{empty}$ mass difference is then $\sqrt{0.000006^2 + 0.000006^2} = 0.000008 \approx 0.00001$ g.

b) $\bar{x}$: mean; s: standard deviation; $\bar{u}$: pooled standard uncertainty of individual values; $u(\bar{x})$: standard uncertainty of the mean, $u(\bar{x}) = \sqrt{(s^2 + \bar{u}^2)/3}$.

The density of SRM 967b High Level at the reference 23 °C is therefore:

$$\rho_{23\text{serum}} = \left(\frac{0.52098 \pm 0.00007}{0.50766 \pm 0.00005}\right)\left(\frac{1 - (0.00021 \pm 0.00001)(19.54 \pm 0.01 - 23)}{1 - (0.00021 \pm 0.00001)(19.79 \pm 0.01 - 23)}\right)(0.99756 \pm 0.00001)$$
$$= (1.02379 \pm 0.00020)\frac{\text{g}}{\text{mL}}.$$

For two degrees of freedom, the Student's t_{95} expansion factor, k_{95}, is 4.3, for an approximate 95 % level of confidence expanded uncertainty interval, $\rho_{23\text{serum}} \pm U_{95}$, of:

$$\rho_{23\text{serum}} \pm U_{95} = \rho_{23\text{serum}} \pm k_{95}u = 1.02379 \pm (4.3)(0.00020) = (1.02379 \pm 0.00086)\frac{\text{g}}{\text{mL}}$$

6. Creatinine Molecular Weight

The molecular formula for creatinine is $C_4H_7N_3O$. Using the IUPAC Molecular Weight calculator [15,16], the molecular weight (molar mass) of creatinine is (113.1182 ± 0.0025) g/mol. The associated 95 % level of confidence interval is (113.1138 to 113.1227) g/mol. The calculator combines elemental atomic weights assuming that the weights are normally distributed across their stated uncertainty intervals; Fig. 13 displays the resulting probability density for creatinine.

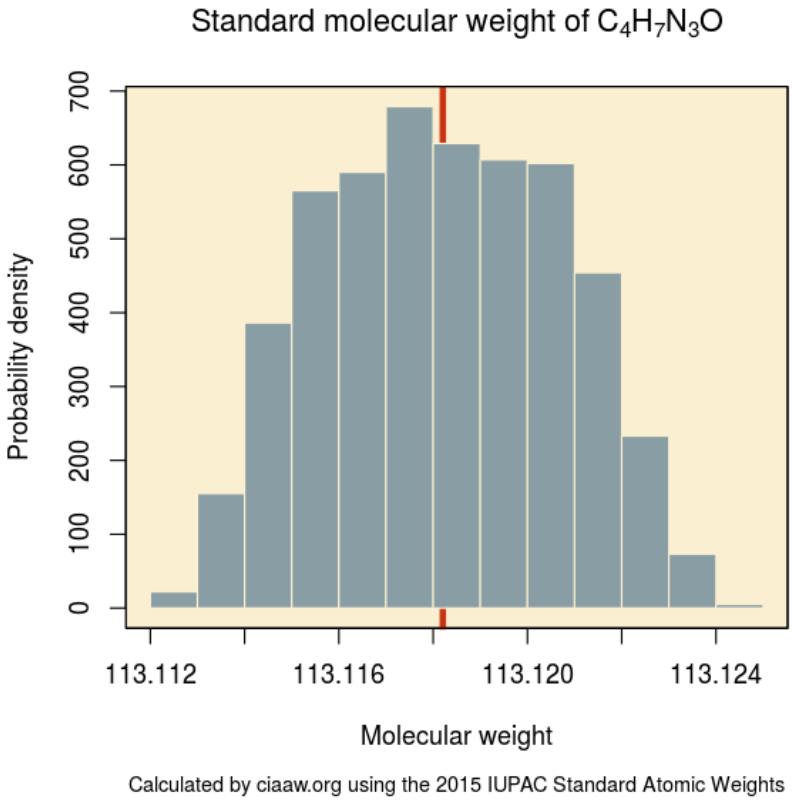

Fig. 13. Probability Density for the Molecular Weight of Creatinine.

7. Statistical Analysis

Assignment of mass fraction (µg/g), mass concentration (mg/dL) and amount-of-substance concentration (mmol/L) of creatinine in human serum proceeded in stages. The mass fractions are first assigned using the ID-LC-MS results described in Section 4. Mass concentration is then assigned by combining the mass fraction estimates with the experimentally determined serum densities described in Section 5. Amount of substance concentration is then assigned by combining the mass concentration estimates with the creatinine molecular weight (molar mass) assessment described in Section 6.

7.1. Assessment of Mass Fraction

The mass fraction measurements were made at two levels (high and low), over 4 days. There were 4 calibrants, each measured twice. The same calibration data was used for the two levels.

The analysis was done using an OpenBUGS [17] implementation of an internal standard-response model derived from that used in the NIST "Apps for Bayesian Analysis of Chemical quantities Using Shiny)" (ABACUS) package [12]. This model pools the injection values toward the box values and towards the grand mean of all samples within the level. The uncertainty in the grand mean is increased to accommodate any small apparent measurement differences among the boxes, aliquots, and injections. The use of the model is predicated upon the assumption that all of the input data has been critically evaluated and found to be adequately homogeneous. The results presented in Fig. 11 suggest that all results for both levels are valid.

The model and its inputs are provided in Section 7.1.1. For convenience, Table 17 echoes the model's required mass fraction working standard information provided in Table 7, Table 18 extracts the mass and peak area ratio calibration solution information provided in Table 8 and Table 9, and Table 19 extracts the mass and peak area ratio sample solution information provided in Table 13 and Table 14. When not otherwise specified the model assumes mass determinations have an associated uncertainty of 0.000015 g.

Table 17. Mass Fraction of Creatinine in Working Standard Solutions.

	Mass Fraction, µg/g	
Std	wadm	uwad
1	15.5516	0.0078
2	15.6669	0.0079
3	12.8656	0.0065
4	13.1432	0.0066

Std: working standard index.
wadm: mass fraction of creatinine in a working standard solution, labeled $w_{analyte}$ in Table 7
uwad: estimated uncertainty in the mass fraction estimate, labeled $u(w_{analyte})$ in Table 7

Table 18. Calibration Sample Information.

Set	Std	mid	mad	rac1	rac2
1	1	0.17650	0.12072	0.77395	0.76852
1	2	0.17502	0.13516	0.85894	0.86504
1	3	0.17469	0.18531	0.95836	0.95990
1	4	0.17468	0.21948	1.16773	1.16338
2	1	0.17274	0.12160	0.78767	0.78887
2	2	0.17543	0.13635	0.90229	0.87698
2	3	0.17488	0.18549	0.97089	0.96169
2	4	0.17514	0.22082	1.20780	1.20540
3	1	0.17318	0.12264	0.80151	0.79092
3	2	0.17487	0.13574	0.88976	0.88313
3	3	0.17501	0.18580	0.98491	0.97505
3	4	0.17496	0.21919	1.18490	1.18187
4	1	0.17349	0.12186	0.79234	0.77867
4	2	0.17356	0.13589	0.87861	0.87309
4	3	0.17375	0.18463	0.99044	1.00268
4	4	0.17481	0.21862	1.18983	1.17266

Set: measurement set index. All results within a set were obtained on the same day.
Std: working standard index. All calibrants with the same index were prepared from the same working standard.
mid: mass of internal standard added to calibrant; labeled m_{stockIS} in Table 8
mad: mass of analyte working standard added calibrant; labeled $m_{\text{stockAnalyte}}$ in Table 8
rac1, rac2: peak area ratios for first and second calibrant replicate, labeled "Inj 1" and "Inj 2" in Table 9. These results are entered sequentially in the OpenBUGS code, "rac1" first followed by "rac2".

Table 19. SRM 967b Low and High Level Sample Information.

		SRM 967b Low Level				SRM 967b High Level			
Set	Std	mids	mdi	ras1	ras2	mids	mdi	ras1	ras2
1	1	0.17543	0.36306	1.00378	1.00257	0.17565	0.06275	0.96256	0.96796
1	2	0.17519	0.36408	1.00113	1.00401	0.17524	0.06423	1.00352	0.99532
1	3	0.17505	0.36458	1.01033	1.01373	0.17523	0.06392	0.99213	0.98611
1	4	0.17525	0.36140	1.00205	0.99709	0.17542	0.06390	0.99901	0.99183
2	1	0.17500	0.36493	1.03426	1.02066	0.17488	0.06444	1.02260	1.02545
2	2	0.17498	0.36417	1.02789	1.01826	0.17529	0.06487	1.03742	1.01075
2	3	0.17507	0.36491	1.02097	1.02637	0.17516	0.06389	1.00112	1.01231
2	4	0.17519	0.36236	1.03087	1.02443	0.17516	0.06408	1.00398	1.03166
3	1	0.17513	0.36147	1.01587	1.01777	0.17498	0.06396	1.01603	1.01712
3	2	0.17525	0.35936	1.00777	1.01127	0.17484	0.06406	1.01145	1.01005
3	3	0.17517	0.35906	1.02113	1.01797	0.17500	0.06466	1.02512	1.01708
3	4	0.17492	0.35769	1.01006	1.00509	0.17468	0.06388	1.01440	1.01813
4	1	0.17457	0.36119	1.01259	1.00363	0.17447	0.06551	1.02335	1.04086
4	2	0.17436	0.36055	1.01293	1.02744	0.17441	0.06462	1.02149	1.03349
4	3	0.17438	0.36301	1.01837	1.01839	0.17464	0.06412	1.01250	0.99926
4	4	0.17449	0.34763	0.97725	0.96935	0.18542	0.06493	0.97607	0.96170

Set: measurement set index. All results within a set were obtained on the same day.
Std: working standard index. All calibrants with the same index were prepared from the same working standard.
mids: mass of internal standard added to sample; labeled m_{stockIS} in Table 13 and Table 14.
mdi: mass of serum in sample; labeled m_{sample} in Table 13 and Table 14.
ras1, ras2: peak area ratios for first and second sample replicates, labeled "A_{114}/A_{117}" in Table 13 and Table 14. These results are entered sequentially in the OpenBUGS code, "ras1" first followed by "ras2".

7.1.1. OpenBUGS Bayesian Model

```
####################
# Inputs
# MT        number of number of sets × samples/set (4×4)
# MTT       number of sets × samples/set × replicates/sample (4×4×2)
# NS        number sets (4)
# NT        number of sets × working standards (4×4)
# NTT       number of sets × calibrants/set × replicates/calibrant (4×4×2)
# NWS       Number working standards (4)
# wsol      NT index of working standard within set
# Ics0      NT index of set by calibrant
# Ics1      NTT index of set by calibrant by replicate
# Ics2      NTT index of calibrant measurement by replicate
# madu      NS standard uncertainties for working standard masses
# midu      NS standard uncertainties of internal standard masses
# wad       NWS mass fraction of working standards
# wadu      NWS standard uncertainties for working standard mass fractions
# mid       NT internal standard masses added to calibrants
# mad       NT working standard masses added to calibrants
# rac       NTT peak area ratios for calibrants
# Ism0      MT index of set by sample
# Ism1      NTT index of set by sample by replicate
# Ism2      NTT index of sample measurement by replicate
# mids      MT internal standard masses in samples
# mdi       MT serum mass in sample
# ras       MTT peak area ratios for samples
#
####################
# Output
# MFtotal   1 mass fraction analyte
#
####################
# Intermediates
# b         NS calibration slope distributions
# b.cut     NS prevents feedback from samples
# chsqns    NS
# chsqnsi   NS
# chsqnsw   NS
# fitp      NS lack-of-fit variance of the statistical model
# i         1 index
# madd      NT distributions for working standards
# madp      NS precisions for working standards
# mean      NTT
# MFreps    MTT replicate mass fractions
# MFset     NS set mass fractions
# midd      NT distributions for internal standard masses
# midp      NS precisions for internal standard masses
# rasmean   MT
# rasmeanp  MT
# rassig    NS between replicates within a sample variance
# ratio     NT
# wac       NT
# wadd      NWS distributions for working standards
# wadp      NWS precisions for working standards
# xins      NS
# xinsi     NS
# xinsw     NS
# wdsig     NS
# T         1 used in linear pool
# P         NS used in linear pool
# alpha     NS used in linear pool
#
```

```
####################
# Code begins here
####################
Model{
# calibrant setup
for(i in 1:NWS){
  wadp[i]<-1/(wadu[i]*wadu[i])
  wadd[i]~dnorm(wad[i],wadp[i])}
for(i in 1:NS){
  midp[i]<-1/(midu[i]*midu[i])
  madp[i]<-1/(madu[i]*madu[i])}
for(i in 1:NT){
  madd[i]~dnorm(mad[i],madp[Ics0[i]])
  midd[i]~dnorm(mid[i],midp[Ics0[i]])
  ratio[i]<-madd[i]/midd[i]
  wac[i]<-wadd[wsol[i]]* ratio[i]}
#
# calibration equation
# b[i] is the RF of Eq. 1 per each calibrant
# fitp[i] is the lack-of-fit variance of the statistical model (Eqs. 1-3) to the data
for(i in 1:NS){
  b[i]~dnorm(0,1.0E-5)
  xins[i]~dnorm(0,0.0016)I(0.001,)
  chsqns[i]~dgamma(0.5,0.5)
  fitp[i]<-xins[i]/sqrt(chsqns[i])}
#
for(i in 1:NTT){
  mean[i]<-b[Ics1[i]]*wac[Ics2[i]]
  rac[i]~dnorm(mean[i],fitp[Ics1[i]])}
#
# Compute the sample mass fractions
# rassiq[i] is the between replicates within a sample variance
# MFset[i] is the pooled mass fraction as in Eq. 3, over samples in a set
for(i in 1:NS){
  b.cut[i]<-cut(b[i])
  xinsi[i]~dnorm(0,0.0016)I(0.001,)
  chsqnsi[i]~dgamma(0.5,0.5)
  rassig[i]<-xinsi[i]/sqrt(chsqnsi[i])
  MFset[i]~dnorm(0,1.0E-5)}
#
# Calculate MFset[i] using Eqs. 2 and 3, solved for peak area ratio ras[i]
for(i in 1:MT){
  rasmean[i]<-b.cut[Ism0[i]]* MFset[Ism0[i]]*mdi[i]/mids[i]
  rasmeanp[i]~dnorm(rasmean[i],fitp[Ism0[i]])}
for(i in 1:MTT){ras[i]~dnorm(rasmeanp[Ism2[i]],rassig[Ism1[i]])}
#
# Combine using linear pool
for(i in 1:NS){alpha[i]<-1}
P[1:NS]~ddirich(alpha[])
T~dcat(P[])
MFtotal<-MFset[T]
} End of model
```

7.1.1.1. Calibration Data

```
####################
# Data for calibrants
# Load for analysis of Low and High Levels
####################
list(
MT=16,MTT=32,NS=4,NT=16,NTT=32,NWS=4,
wsol=c(1,2,3,4,1,2,3,4,1,2,3,4,1,2,3,4),
Ics0=c(1,1,1,1,2,2,2,2,3,3,3,3,4,4,4,4),
Ics1=c(1,1,1,1,2,2,2,2,3,3,3,3,4,4,4,4,
       1,1,1,1,2,2,2,2,3,3,3,3,4,4,4,4),
Ics2=c(1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,
       1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16),
madu=c(0.000015,0.000015,0.000015,0.000015),
midu=c(0.000015,0.000015,0.000015,0.000015),
wad=c(15.5516,15.6669,12.8656,13.1432),
wadu=c( 0.0078,0.0079,0.0065,0.0066),
mid=c(0.17650,0.17502,0.17469,0.17468,0.17274,0.17543,0.17488,0.17514,
      0.17318,0.17487,0.17501,0.17496,0.17349,0.17356,0.17375,0.17481),
mad=c(0.12072,0.13516,0.18531,0.21948,0.12160,0.13635,0.18549,0.22082,
      0.12264,0.13574,0.18580,0.21919,0.12186,0.13589,0.18463,0.21862),
rac=c(0.77395,0.85894,0.95836,1.16773,0.78767,0.90229,0.97089,1.20780,
      0.80151,0.88976,0.98491,1.18490,0.79234,0.87861,0.99044,1.18983,
      0.76852,0.86504,0.95990,1.16338,0.78887,0.87698,0.96169,1.20540,
      0.79092,0.88313,0.97505,1.18187,0.77867,0.87309,1.00268,1.17266))
```

7.1.1.2. SRM 967b Low Level Sample Set Data

```
####################
# Data for Low Level samples
# Load only for analysis of Low samples
####################
list(
Ism0=c(1,1,1,1,2,2,2,2,3,3,3,3,4,4,4,4),
Ism1=c(1,1,1,1,2,2,2,2,3,3,3,3,4,4,4,4,
       1,1,1,1,2,2,2,2,3,3,3,3,4,4,4,4),
Ism2=c(1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,
       1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16),
mids=c(0.17543,0.17519,0.17505,0.17525,0.17500,0.17498,0.17507,0.17519,
       0.17513,0.17525,0.17517,0.17492,0.17457,0.17436,0.17438,0.17449),
mdi=c(0.36306,0.36408,0.36458,0.36140,0.36493,0.36417,0.36491,0.36236,
      0.36147,0.35936,0.35906,0.35769,0.36119,0.36055,0.36301,0.34763),
ras=c(1.00378,1.00113,1.01033,1.00205,1.03426,1.02789,1.02097,1.03087,
      1.01587,1.00777,1.02113,1.01006,1.01259,1.01293,1.01837,0.97725,
      1.00257,1.00401,1.01373,0.99709,1.02066,1.01826,1.02637,1.02443,
      1.01777,1.01127,1.01797,1.00509,1.00363,1.02744,1.01839,0.96935))
```

7.1.1.3. SRM 967b High Level Sample Set Data

```
####################
# Data for High Level samples
# Load only for analysis of High samples
####################
list(
Ism0=c(1,1,1,1,2,2,2,2,3,3,3,3,4,4,4,4),
Ism1=c(1,1,1,1,2,2,2,2,3,3,3,3,4,4,4,4,
       1,1,1,1,2,2,2,2,3,3,3,3,4,4,4,4),
Ism2=c(1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,
       1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16),
mids=c(0.17565,0.17524,0.17523,0.17542,0.17488,0.17529,0.17516,0.17516,
       0.17498,0.17484,0.17500,0.17468,0.17447,0.17441,0.17464,0.18542),
mdi=c(0.06275,0.06423,0.06392,0.06390,0.06444,0.06487,0.06389,0.06408,
      0.06396,0.06406,0.06466,0.06388,0.06551,0.06462,0.06412,0.06493),
ras=c(0.96256,1.00352,0.99213,0.99901,1.02260,1.03742,1.00112,1.00398,
      1.01603,1.01145,1.02512,1.01440,1.02335,1.02149,1.01250,0.97607,
      0.96796,0.99532,0.98611,0.99183,1.02545,1.01075,1.01231,1.03166,
      1.01712,1.01005,1.01708,1.01813,1.04086,1.03349,0.99926,0.96170))
```

7.2. Mass Fraction

Table 20 lists the creatinine mass fractions in the SRM 967b Low and High Level materials as estimated using the OpenBUGS model and data provided in Section 7.1.1. The posterior distributions of the estimates are displayed in Fig. 14.

Table 20. Creatinine Mass Fraction in SRM 967b Low and High Levels.

	Mass Fraction, µg/g					%
SRM 967b	$w_{analyte}$	$u(w_{analyte})$	$w_{2.5\%}$	$w_{97.5\%}$	$U_{95}(w_{analyte})$	$u_{rel}(w_{analyte})$
Low Level	6.825	0.072	6.680	6.965	0.144	1.05
High Level	38.35	0.44	37.47	39.24	0.88	1.15

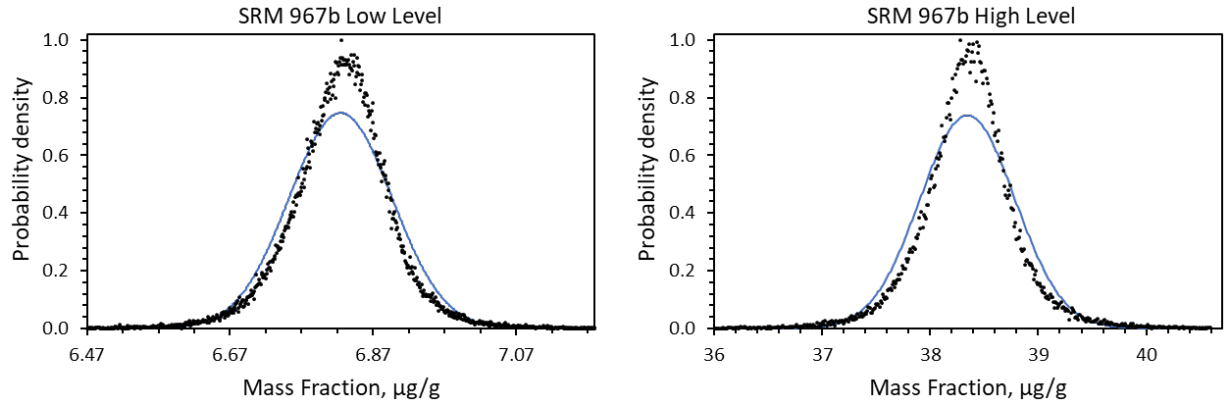

Fig. 14. Posterior Distributions for Creatinine Mass Fraction in the SRM 967b Materials

The dots represent the probability density of the OpenBUGS model's creatinine mass fraction results, estimated from 100 000 samples drawn from solutions using the input data values, uncertainties, and assumed distributions. The solid lines represent Gaussian distributions having the estimated means and standard deviations.

7.2.1. Homogeneity

To demonstrate homogeneity across vials, Fig. 15 displays the measurement results as a function of the vial number. Relative to the estimated $w_{\text{analyte}} \pm U_{95}(w_{\text{analyte}})$ intervals, there is no evidence of between-vial heterogeneity.

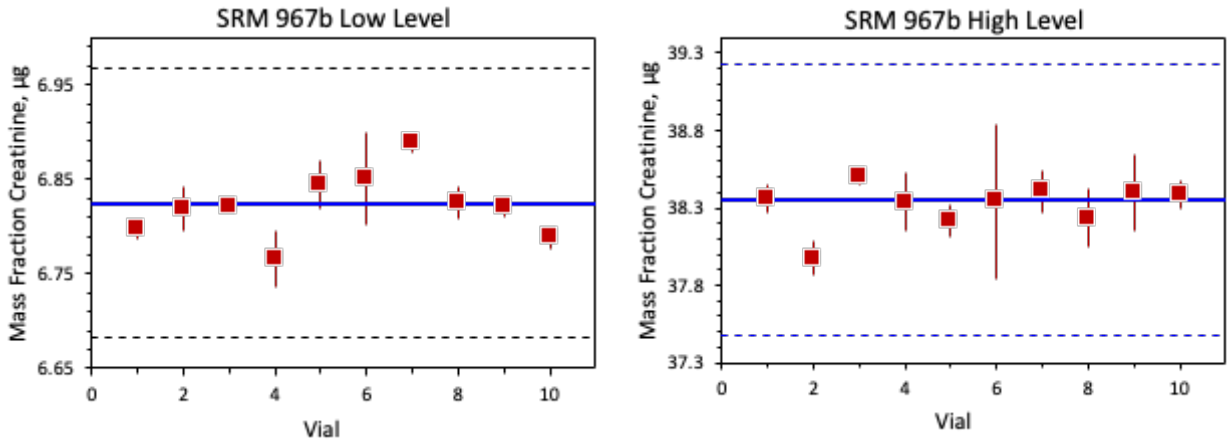

Fig. 15. SRM 967b Measurement Results as a Function of Vial Number.

Results for SRM 967b Low Level (left) and High Level (right) vials plotted in as a function of vial number. The solid squares represent the mean of the measurement results for each vial; the error bars represent one standard uncertainty of the mean. The solid horizontal lines represent the estimated w_{analyte}; the dashed lines bound the $w_{\text{analyte}} \pm U_{95}(w_{\text{analyte}})$ intervals.

7.3. Mass Concentration

The mass fraction results expressed are converted into mass concentration through multiplication by the serum density at the reference temperature, 23°C:

$$\gamma_{\text{analyte}}\,\frac{\text{mg}}{\text{dL}} = \left(w_{\text{analyte}}\,\frac{\mu\text{g}}{\text{g}}\right)\left(\rho_{\text{serum}}\,\frac{\text{g}}{\text{mL}}\right)\left(\frac{\text{mg}}{1000\ \mu\text{g}}\right)\left(\frac{100\ \text{mL}}{\text{dL}}\right) \tag{10}$$

Table 21 lists the creatinine mass concentrations in the SRM 967b Low and High Level materials as estimated using the NIST Uncertainty Machine (NUM) [18] to combine the creatinine mass fractions determined above with the serum densities estimated in Section 5. The probability densities for the mass concentration estimates are displayed in Fig. 16.

Table 21. Creatinine Mass Concentration in SRM 967b Low and High Levels.

	, Mass Concentration, mg/dL					%
SRM 967b	w_{analyte}	$u(w_{\text{analyte}})$	$w_{2.5\%}$	$w_{97.5\%}$	$U_{95}(w_{\text{analyte}})$	$u_{\text{rel}}(w_{\text{analyte}})$
Low Level	0.697	0.0075	0.6827	0.7116	0.0150	1.07
High Level	3.926	0.045	3.838	4.015	0.090	1.15

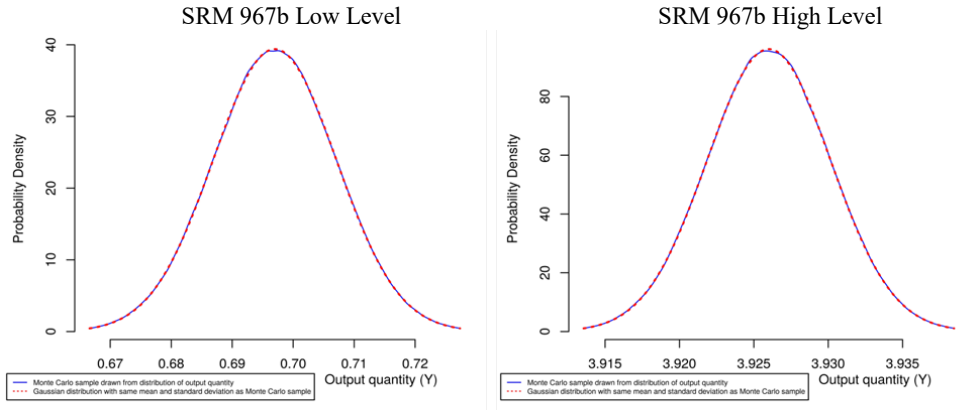

Fig. 16. NIST Uncertainty Machine Estimates of Mass Concentration.

The curves display the estimated probability density distributions for the determination of mass concentration from mass fraction and serum density. The solid curves represent the density of a Monte Carlo sample drawn from the distribution of the product of the two terms, the superimposed dotted curves represent Gaussian distributions having the same means and standard deviations.

7.4. Amount-of-Substance Concentration

The amount concentration results expressed are converted into amount-of-substance concentration through division by the molecular weight (molar mass) of creatinine:

$$c_{\text{analyte}}\,\frac{\text{mmol}}{\text{L}} = \left(\gamma_{\text{analyte}}\,\frac{\text{mg}}{\text{dL}}\right)\left(\frac{10\ \text{dL}}{\text{L}}\right)\left(\frac{\text{g}}{1000\ \text{mg}}\right)/\left(M_{\text{analyte}}\,\frac{\text{g}}{\text{mol}}\right)\left(\frac{\text{mol}}{1000\ \text{mmol}}\right) \tag{11}$$

Table 22 lists the creatinine amount-of-substance concentrations in the SRM 967b Low and High Level materials as estimated using the NUM [18] to combine the creatinine mass concentrations determined above with the molecular weight as estimated in Section 6. The probability densities for the amount-of-substance concentration estimates are displayed in Fig. 17.

Table 22. Creatinine Amount-of-Substance Concentration in SRM 967b Low and High Levels.

	Amount of Substance Concentration, mmol/L					%
SRM 967b	c_{analyte}	$u(c_{\text{analyte}})$	$c_{2.5\%}$	$c_{97.5\%}$	$U_{95}(c_{\text{analyte}})$	$u_{\text{rel}}(c_{\text{analyte}})$
Low Level	0.06163	0.00066	0.6035	0.6290	0.00132	1.07
High Level	0.3471	0.00398	3.393	3.549	0.00796	1.15

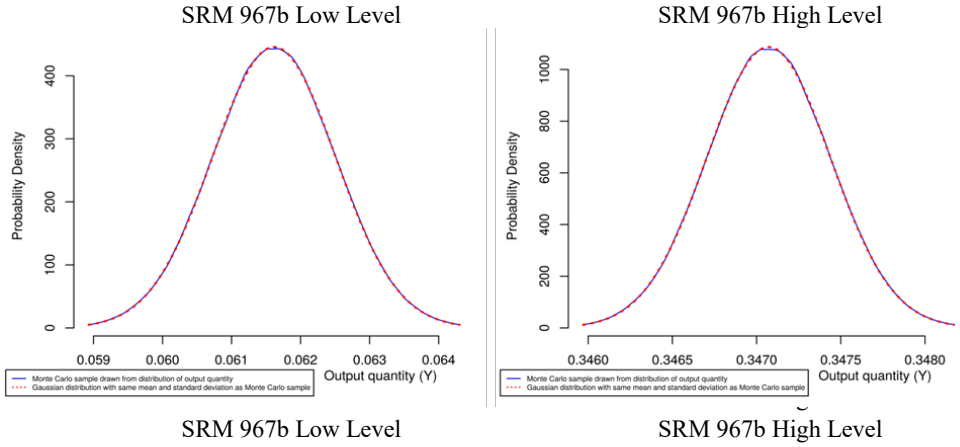

Fig. 17. NIST Uncertainty Machine Estimates of Amount-of-Substance Concentration.

The curves display the estimated probability density distributions for the determination of amount-of-substance concentration from mass concentration and the molecular weight of creatinine. The solid curves represent the density of a Monte Carlo sample drawn from the distribution of the product of the two terms, the superimposed dotted curves represent Gaussian distributions having the same means and standard deviations.

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Appendix A. List of Symbols, Abbreviations, and Acronyms

A.1. Acronyms

ABACUS	Apps for Bayesian Analysis of Chemical quantities Using Shiny
CV	coefficient of variation expressed as a percentage
FWA	Federal-wide Assurance
HPLC	high performance liquid chromatography
ID	isotope dilution
IRB	Institutional Review Board
IS	internal standard
IS-LC-MS	internal standard liquid chromatography mass spectrometry
JCTLM	Joint Committee for Traceability in Laboratory Medicine
LC	liquid chromatography
LC-MS	liquid chromatography mass spectrometry
lsrd	least-significant reported digit
MS	mass spectrometry
NIST	National Institute of Standards and Technology
NUM	NIST Uncertainty Machine
OHRP	United States Department of Health and Human Services' Office for Human Research Protections
PDVF	polyvinylidene difluoride
RMP	reference measurement procedure
RF	response factor
SD	standard deviation
SI	International System of Units (Système international d'unités)
SIM	selected ion monitoring
SRM®	Standard Reference Material®
TPOC	Technical Point of Contact

A.2. Symbols

$(\cdot)_{2.5\%}$	the 2.5 % percentile of the probability density for "(·)"
$(\cdot)_{97.5\%}$	the 97.5 % percentile of the probability density for "(·)"
$\rho_{\mathrm{water},T}$	density (g/mL) of water at temperature T
$u(\cdot)$	standard uncertainty of value denoted by "(·)"
$u_{\mathrm{rel}}(\cdot)$	relative standard uncertainty of value denoted "(·)"
$U_{95}(\cdot)$	expanded uncertainty at the 95 % level of confidence
R	response factor
$A_{(\cdot)}$	area of a peak for component "(·)"
$m_{(\cdot)}$	mass of component "(·)"
$\gamma_{(\cdot)}$	mass concentration (g/dL) of "(·)"
$c_{(\cdot)}$	amount-of-substance concentration (mol/m^3) of "(·)"
$w_{(\cdot)}$	mass fraction (g/g) of "(·)"
$p_{(\cdot)}$	purity (g/g) of "(·)"
$\rho_{(\cdot)}$	density (g/mL) of s f "(·)"
T	Temperature in °C
m/z	mass-to-charge ratio