NIST Special Publication 260
NIST SP 260-253

Certification of Standard Reference Material® 965c Glucose in Frozen Human Serum

Amanda Bayless
W. Clay Davis
Elena S.C. Wood
Abraham Kuri-Cruz
Johanna Camara
Blaza Toman
Komal Dahya
Katherine R. Earl
Chui Y. Tse
Uliana Danilenko
Hubert W. Vesper

This publication is available free of charge from:
https://doi.org/10.6028/NIST.SP.260.253

NIST NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY
U.S. DEPARTMENT OF COMMERCE

Page Intentionally Blank

NIST Special Publication 260
NIST SP 260-253

Certification of Standard Reference Material® 965c Glucose in Frozen Human Serum

Amanda Bayless, W. Clay Davis, Elena S.C. Wood, Abraham Kuri-Cruz, Johanna Camara
Chemical Sciences Division
Material Measurement Laboratory
National Institute of Standards and Technology

Blaza Toman
Statistical Engineering Division
Information Technology Laboratory
National Institute of Standards and Technology

Komal Dahya, Katherine R. Earl, Chui Y. Tse, Uliana Danilenko, Hubert W. Vesper
CDC Clinical Reference Laboratory
Division of Laboratory Sciences
National Center for Environmental Health

This publication is available free of charge from:
https://doi.org/10.6028/NIST.SP.260.253

March 2025

U.S. Department of Commerce
Howard Lutnick, Secretary

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

NIST SP 260-253
March 2025

Certain equipment, instruments, software, or materials, commercial or non-commercial, are identified in this paper in order to specify the experimental procedure adequately. Such identification does not imply recommendation or endorsement of any product or service by NIST, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of Centers for Disease Control and Prevention. Use of trade names and commercial sources is for identification only and does not constitute endorsement by the Centers for Disease Control and Prevention or the U.S. Department of Health and Human Services.

Publication History
Approved by the NIST Editorial Review Board on 2025-03-13

How to Cite this NIST Technical Series Publication
Bayless A, Davis WC, Wood ESC, Kuri-Cruz A, Camara J, Toman B, Dahya K, Earl KR, Tse CY, Danilenko U, Vesper HW (2025) Certification of Standard Reference Material® 965c Glucose in Frozen Human Serum. (National Institute of Standards and Technology, Gaithersburg, MD), NIST Special Publication 260 (SP) NIST SP 260-253. https://doi.org/10.6028/NIST.SP.260-253.

Author ORCID iDs

Bayless A	0000-0002-9073-5582
Camara J	0000-0002-9415-8452
Danilenko U	0000-0002-4718-5626
Dahya K	0000-0002-2483-1568
Davis WC	0000-0001-9076-2620
Earl KR	0009-0008-9792-5718
Kuri Cruz A	0000-0003-4409-0653
Toman B	0000-0002-0999-9565
Tse CY	0000-0002-5417-1599
Vesper HW	0000-0002-9649-9015
Wood ESC	0009-0000-8537-3006

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

Abstract

Standard Reference Material (SRM) 965c Glucose in Frozen Human Serum is intended for use in validating methods for the determination of glucose in human serum and plasma. A unit of SRM 965c consists of three ampoules each of four materials: Level 1, Level 2, Level 3, and Level 4. Each ampoule contains approximately 1.0 mL of frozen serum. The value assignment of SRM 965c was a collaboration between the National Institute of Standards and Technology (NIST) and the Division of Laboratory Sciences, Centers for Disease Control and Prevention (CDC). This publication documents the production, analytical methods, and computations involved in characterizing this product.

Keywords

Glucose; Isotope Dilution Gas Chromatography Mass Spectrometry (ID-GC-MS); Isotope Dilution Liquid Chromatography Tandem Mass Spectrometry (ID-LC-MS/MS); Reference Measurement Procedure (RMP); Standard Reference Material (SRM).

Table of Contents

List of Tables

List of Figures

1. Introduction

Glucose is a chiral sugar that exists in solution as an equilibrium mixture of six- and five-member rings and an open chain form, with six-membered rings dominant. The chemical structures of glucose in solution are displayed in Fig. 1 [1].

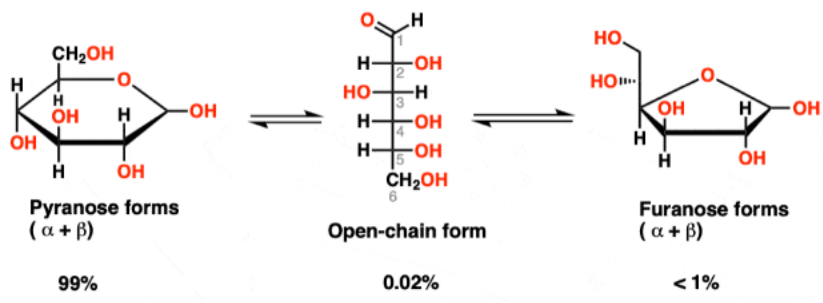

Fig. 1. Glucose Structures in Solution.

Blood glucose monitoring is essential for the clinical management of diabetes mellitus [2]. The National Institute of Standards and Technology (NIST) Standard Reference Material® (SRM®) 965c Glucose in Frozen Human Serum is intended to support the serum glucose measurement processes of *in vitro* diagnostics manufacturers, reference laboratories, and clinical laboratories in the United States and globally.

SRM 965c is the fourth member of the SRM 965 series. The original SRM 965 Glucose was issued in late 1996 [3]. It was followed by SRM 965a in 2004 [4] and SRM 965b in 2009 [5]. The sales history of these materials is displayed in Fig. 2. More than 3400 units of the SRM 965 series have been sold. The proportion of these sales to various geographical regions is displayed in Fig. 3. Early sales were mostly to locations within the United States. Since 2005, about half of sales have been to domestic customers.

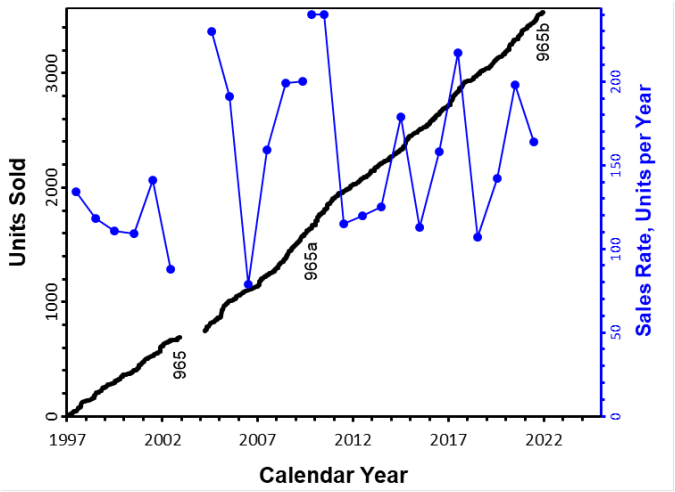

Fig. 2. Sales History of the SRM 965 Series.

The thick black line depicts the cumulative distribution of sales as a function of the invoice date; it is plotted using the "Units Sold" axis at the left. 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.

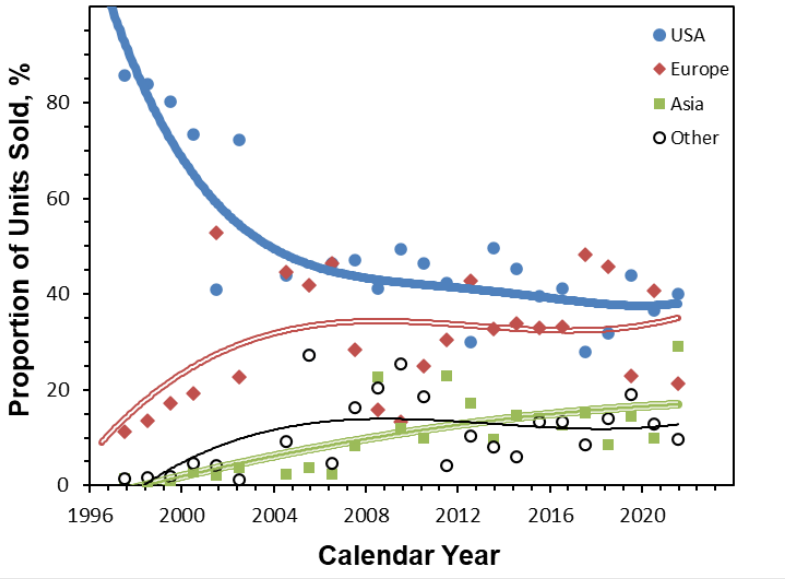

Fig. 3. Location of Customers for the SRM 965 Series.

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

A unit of SRM 965c consists of twelve flame-sealed ampoules of frozen human serum, three ampoules at each of four different glucose concentration levels. Each ampoule contains approximately 1.0 mL of human serum.

The SRM 965c glucose concentrations were assigned by combining NIST isotope dilution (ID)-liquid chromatography (LC)-tandem mass spectroscopy (MS/MS) [6] and Centers for Disease Control and Prevention (CDC) ID-gas chromatography (GC)-mass spectroscopy (MS) [7] results. The NIST ID-LC-MS/MS implementation is based on a method recognized by the Joint Committee for Traceability in Laboratory Medicine (JCTLM) [8] as a reference measurement procedure (RMP). The CDC method was submitted to JCTLM as a candidate RMP for review. The uncertainties of serum glucose values assigned using the JCTLM-listed RMP (NIST) or the candidate RMP submitted for JCTLM listing (CDC) has been proven fit-for-purpose.

Based on experience with the previous members of the SRM 965 series, the glucose levels of the SRM 965c materials are expected to be stable for at least 10 years post-production. Stability will be evaluated within one year of the expiration date, with intermittent monitoring through its use as a control whenever NIST makes glucose in serum measurements.

Metrological traceability to the International System of Units (SI) of the assigned glucose values in the SRM 965c materials is through calibration with SRM 917d D-Glucose (Dextrose) [9].

2. Materials, Production, and Verification

A contract was awarded for the preparation of 6000 ampoules each of four levels of SRM 965c Glucose in Frozen Human Serum.

- Level 1 was prepared from a normal serum pool depleted of glucose with immobilized glucose oxidase/catalase.
- Level 2 and Level 3 were prepared from normal serum pools without augmentation.
- Level 4 was prepared from a normal serum pool with the addition of pure glucose (Dextrose, product number D9434, obtained from Millipore Sigma).

The final SRM 965c materials were delivered on dry ice to the NIST Office of Reference Materials (ORM) and stored at -80 °C. The design and requirements for the SRM materials are described in the following extracts from the Statement of Work (SOW). The verification of the glucose concentrations in the SRM 965c materials is described in Section 2.3.

2.1. Statement of Work

2.1.1. Section I. Background Information

The National Institute of Standards and Technology (NIST) has provided batches of Standard Reference Material (SRM) 965 Glucose in Frozen Human Serum since 1996. This material supports the measurements of in vitro diagnostics manufacturers, reference laboratories, and clinical laboratories in the United States and globally. The current batch of SRM 965b Glucose in Frozen Human Serum is out of stock.

2.1.2. Section II. Specifications

2.1.2.1. Task 1: SRM 965c Level 1

Contractor shall provide 6000 ampoules containing (1.1 ± 0.1) mL pooled human serum from whole blood containing (30-40) mg/dL glucose.

2.1.2.2. Task 2: SRM 965c Level 2

Contractor shall provide 6000 ampoules containing (1.1 ± 0.1) mL pooled human serum from whole blood containing (70-90) mg/dL glucose.

2.1.2.3. Task 3: SRM 965c Level 3

Contractor shall provide 6000 ampoules containing (1.1 ± 0.1) mL pooled human serum from whole blood containing (115-135) mg/dL glucose.

2.1.2.4. Task 4: SRM 965c Level 4

Contractor shall provide 6000 ampoules containing (1.1 ± 0.1) mL pooled human serum from whole blood containing (290-310) mg/dL glucose.

2.1.2.5. Task 5: Ampoule Stress Test Results

The contractor shall provide the NIST Technical Point of Contact (TPOC) with ampoule stress test results prior to filling the ampoules.

2.1.2.6. Task 6: Certificate of Analysis

The contractor shall provide the TPOC with the Certificate of Analysis, including pilot/validation study, prior to shipping the ampoules to NIST.

2.1.2.7. Task 7: Material Preparation Detail Report

The contractor shall provide the TPOC with the material preparation report, including the source of the added glucose (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.

The NIST Report of Analysis is an internal NIST report. NIST is starting a new process whereby information is compiled from these internal reports and edited for eventual presentation as an external NIST Special Publication.

NIST intends to publish the contractor's responses to the solicitation and any Certificates of Analysis provided by the contractor. NIST does not intend to publish proprietary information and will work with the contractor to further redact information from internal NIST reports to assemble the related external Special Publication.

2.1.2.8. Task 8: Biosafety Report in accordance with the requirements herein

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 (https://www.accessdata.fda.gov/scripts/cdrh/devicesatfda/index.cfm).

The contractor shall provide a written Biosafety Report stating the negative results of all single-donor serum units utilized for SRM 965c preparation. Donor units testing positive for any of the stated infectious agents shall 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.

The contractor shall provide a total of 24 000 ampoules of (1.1 ± 0.1) mL per ampoule of four (4) different concentration levels, 6000 ampoules per concentration level. The (1.1 ± 0.1) mL aliquot of serum must be dispensed into 2 mL pre-scored glass ampoules. The 1.1 mL aliquot of serum and 2 mL ampoule size deviates from the container requirement that is specified in section A2.5(2) of CLSI C29-A2 (https://clsi.org/standards/products/clinical-chemistry-and-

toxicology/documents/c29/). The adjusted container requirement MUST be strictly adhered to or breakage of the glass ampoules under –70 °C storage conditions will result.

Only native human serum (not plasma) that has been tested by the contractor and found negative for HIV and hepatitis shall be used. The values of sodium, potassium, and other analytes in this pooled sera must be within the usual reference intervals for a "healthy" adult population, as defined in CLSI C29-A2, Table A1 (https://clsi.org/standards/products/clinical-chemistry-and-toxicology/documents/c29/). The addition of gentamicin sulfate is required for long term storage of this material and mixed into the base pool to give a final concentration of 50 mg/L. Specimens shall be drawn and processed and frozen within 8 hours of the time of collection.

The contractor shall follow the guidelines set forth for base pool specifications as detailed in CLSI Document C29-A2, Appendix A (https://clsi.org/standards/products/clinical-chemistry-and-toxicology/documents/c29/) for the preparation of this four-level glucose in serum material. The serum must be defibrinated or off clot, filtered, and partitioned into four (4) volumes. The target glucose concentration levels are given in the Specific Tasks above.

Spike one or both of the normal levels to achieve the high-level glucose, spiking and depleting as necessary to achieve the desired glucose levels. Blending must be done such that the levels will show no greater variability than 1 % sample to sample in analyte concentration. The serum shall be filled into pre-labeled ampoules (labels will be provided to the contractor by NIST) flushed and sealed under an inert gas, and frozen according to the specifications given in Appendix A2.5 of CLSI Document C29-A2 (https://clsi.org/standards/products/clinical-chemistry-and-toxicology/documents/c29/).

The containers must be Wheaton, 2 mL Cryule, Clear borosilicate glass that conforms to USP Type 1 and ASTM E 438 Type I, Class A requirements, diameter 11mm, height 70 mm, Prescored, Gold Band, Wheaton Item #651486 or equivalent.

The contractor must stress test the lot of serum ampoules prior to filling by picking 10 ampoules 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 ampoule filling.

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

Ampoules shall be transferred, in fill order, from the bottling equipment to a box with ampoule dividers in a "Z" pattern, filling each row left to right. The location of the first bottle in each box shall be noted on each side of the outside corner of the box, and boxes must 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 Monday federal holiday. In addition, the contractor must notify the Contracting Officer's Representative (COR), 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 ampoules shall be shipped to: [Redacted]

A pilot/validation run shall be performed after ampoule filling and prior to shipment of ampoules to NIST. The Contractor shall provide NIST with a Certificate of Analysis indicating glucose concentrations by standard clinical methods (direct hexokinase is acceptable) from 10 ampoules randomly selected by the Contractor from each level to be shipped to NIST for inspection and analysis.

Measurement of the glucose concentrations of representative samples from each level by standard clinical methods is required for acceptance. The target values shall be within 5% of those required herein. The results shall be provided to NIST before shipment of the entire production lot.

2.1.3. Section III. Deliverables and Deliverable Due Dates

The contractor shall deliver the serum and biosafety report to NIST, using Freight on Board (FOB) Destination shipping terms no later than 21 calendar days after the receipt of the labels provided by NIST. However, due to current access restrictions, delivery shall not take place until the contractor coordinates with the designated TPOC and Contracting Officer (CO).

2.1.4. Deliverables and Deliverable Due Dates

2.1.4.1. Biosafety Report

Due Date: 48 hours prior to shipment

2.1.4.2. Ampoule Stress Test Results

Due Date: Prior to ampoule filling

2.1.4.3. Material Preparation Detail Report

Due Date: 48 hours prior to shipment

2.1.4.4. Notification of Shipment

Due Date: 48 hours prior to shipment

2.1.4.5. Certificate of Analysis

Due Date: 48 hours prior to shipment

2.1.4.6. Level 1 SRM – 6000 ampoules

Due Date: 180 days from the date of award

2.1.4.7. Level 2 SRM – 6000 ampoules

Due Date: 180 days from the date of award

2.1.4.8. Level 3 SRM – 6000 ampoules

Due Date: 180 days from the date of award

2.1.4.9. Level 4 SRM – 6000 ampoules

Due Date: 180 days from the date of award

2.1.5. Section V. 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. 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.

2.1.6. Section VI. Acceptable Quality Level

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

2.1.7. Section VII. Monitoring Method

The NIST TPOC will verify that the materials were successfully prepared and acceptable. Acceptance will be based upon the delivery of 6000 ampoules of each pooled serum material (24 000 ampoules total) intact and unbroken, accompanied by screening results for glucose

which indicate that the four levels of material possess glucose levels within specified target ranges. Verification and acceptance will be completed no later than 30 days after receipt of the ampoules at NIST.

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

2.1.8. Section VIII. Minimum Contractor Qualifications

- Documented compliance with ISO 13485:2016 “Medical devices -- Quality Management Systems -- Requirements for Regulatory Purposes”; and
- Experience in analyzing the compounds in section III of this SOW in human serum.

Documentation must be a certificate from ISO or a certificate from a certification organization.

2.1.9. Section VIII. Rights In Data

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

2.2. Contractor's Certificate of Analysis

SOLOMONPARK
Improving the health of the Human Race

CERTIFICATE OF ANALYSIS

Client:	**NIST**
Product:	**Glucose 1-4 SRM 965c**
Lot Number:	**4310**
Production Date:	**Level 1: 02/02/2023** **Level 2: 12/07/2023** **Level 3: 11/30/2022** **Level 4: 01/19/2023**
Expiration Date:	**To Be Determined by Client**
Source:	**Frozen Human Serum**
Form:	**6000 x 1.1 ± 0.1 mL per pool**
Final Values:	**Level 1: 34.5 mg/dL** **Level 2: 91.2 mg/dL** **Level 3: 122.7 mg/dL** **Level 4: 292.7 mg/dL**
Testing:	**AU480 Chemistry Analyzer**
Preservative:	**Gentamicin Sulfate (concentration of 50 mg/L)**
Storage:	**Keep at –70°C**
Shipping Temperature:	**Frozen (on dry ice)**
Homogeneity Testing Complete	**Pools Level 1-4 are Homogenous**

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

2.3. Verification Analysis

2.3.1. Materials.

Single ampoules of each of the four levels of SRM 965b Glucose in Frozen Human Serum were used as control. Three ampoules of each of the four levels of SRM 965c Glucose in Frozen Human Serum (Level 1 to Level 4) were obtained from ORM. For each level, the SRM 965c ampoules were chosen from boxes 1 (first), 38 (middle), and 75 (last). All ampoules were stored at -80 °C prior to use.

2.3.2. Sample Preparation and Instrumental method

A Piccolo Xpress Chemistry Analyzer (Abaxis Global Diagnostics, version 2.1.57) was used in accordance with the procedures provided by the manufacturer. SRM 965b and SRM 965c sera were removed from -80 °C storage and allowed to thaw at room temperature for 30 min before vortex mixing. For each analysis, 100 µL of serum was loaded onto a Comprehensive Metabolic Panel disposable rotor previously stored at 4 °C. Rotors were left on a bench until they reached room temperature for 20 min before loading. The serum ampoules were placed on ice and vortexed between replicate rotor analyses. After addition of serum to the rotor, it was immediately loaded into the Piccolo Xpress analyzer and analyzed. Each analysis took approximately 15 min.

For this glucose screening assessment, one ampoule of each level of SRM 965b control and three ampoules of each level of SRM 965c were measured in either single or duplicate preparations. SRM 965c ampoules were selected from boxes 1 (first), 38 (middle), and 75 (last) for each of the four levels. SRM material, box number, preparation number, run order, and the blood chemistry results provided by the Piccolo Xpress Chemistry Analyzer are listed in Table 1. While not a complete homogeneity study, the results from ampoules from the first, last, and middle of each batch indicated no apparent trends in glucose or any of the other chemistry based on fill order or run order. Results from the duplicate preparations from individual ampoules from each level did not indicate within-ampoule inhomogeneity.

Table 1. Abaxis Comprehensive Metabolic Panel Blood Chemistry Parameters.

SRM	Box	Preparation	Run Order	Sodium (mmol/L)	Potassium (mmol/L)	Total Carbon Dioxide (mmol/L)	Chloride (mmol/L)	**Glucose (mg/dL)**	Calcium (mg/dL)	Blood Urea Nitrogen (mg/dL)	Creatinine (mg/dL)	Alkaline Phosphatase (U/L)	Alanine Aminotransferase (U/L)	Aspartate Aminotransferase (U/L)	Total Bilirubin (mg/dL)	Albumin (g/dL)	Total Protein (g/dL)
965c-1	1	1	1	140	4.8	25	103	**39**	9.9	16	1.2	73	28	32	0.5	4.1	6.9
	38	1	6	137	4.6	22	101	**39**	9.1	16	1.0	72	18	34	0.5	4.0	6.6
	38	2	7	137	4.6	22	101	**40**	9.1	16	1.0	75	16	35	0.5	4.0	6.7
	75	1	12	138	4.8	25	101	**39**	9.2	16	1.0	73	33	34	0.5	4.0	6.7
:			Mean:	138	4.7	24	102	**39**	9.3	16	1.1	73	24	34	0.5	4.0	6.7
			SD:	1	0.1	2	1	**1**	0.4	0	0.1	1	8	1	0.0	0.0	0.1
965c-2	1	1	2	147	5.0	27	112	**103**	9.8	16	1.2	81	33	33	0.4	4.1	6.9
	1	2	3	145	5.0	26	107	**103**	9.5	15	1.1	80	30	33	0.5	4.1	7.0
	38	1	8	141	4.6	27	107	**99**	9.1	16	1.0	80	27	30	0.5	3.9	6.6
	75	2	13	141	4.6	28	106	**100**	9.3	15	1.1	77	13	30	0.5	3.8	6.6
			Mean:	144	4.8	27	108	**101**	9.4	16	1.1	80	26	32	0.5	4.0	6.8
			SD:	3	0.2	1	3	**2**	0.3	1	0.1	2	9	2	0.1	0.2	0.2
965c-3	1	1	4	139	4.6	27	103	**129**	9.2	15	0.8	74	29	30	0.6	4.0	6.9
	38	1	9	141	4.6	27	102	**128**	8.9	16	1.0	75	30	30	0.5	3.9	6.8
	38	1	14	140	4.6	27	105	**128**	9.4	16	1.0	74	29	29	0.5	4.0	6.8
	75	2	15	139	4.6	28	104	**128**	9.6	16	1.0	74	28	32	0.4	3.9	6.9
:			Mean:	140	4.6	27	104	**128**	9.3	16	1.0	74	29	30	0.5	4.0	6.9
			SD:	1	0.0	1	1	**1**	0.3	1	0.1	1	1	1	0.1	0.1	0.1
965c-4	1	1	5	140	4.4	27	-	**297**	8.9	14	1.2	75	26	28	0.5	3.9	6.6
	38	1	10	141	4.5	28	105	**298**	8.9	14	1.0	72	21	29	0.5	3.9	6.7
	38	2	11	140	4.6	28	106	**298**	9.3	15	1.0	77	19	28	0.5	3.9	6.7
	75	1	16	140	4.4	28	106	**299**	9.3	15	1.1	80	26	29	0.5	4.0	6.7
:			Mean:	140	4.5	28	106	**298**	9.1	15	1.1	76	23	29	0.5	3.9	6.7
			SD:	1	0.1	1	1	**1**	0.2	1	0.1	3	4	1	0.0	0.1	0.1

The glucose results for SRM 965b provided by the Abaxis analyzer are compared to the certified concentrations in Fig. 4. While in adequate agreement to support use in screening the SRM 965c materials, the result for SRM 965b Level 3 is biased somewhat high, and the result for Level 4 is biased quite low. These biases could be based on non-commutability of these materials with this system due to augmented serum pools (diluted or spiked) and/or the presence of preservative (gentamicin sulfate) in the formulations.

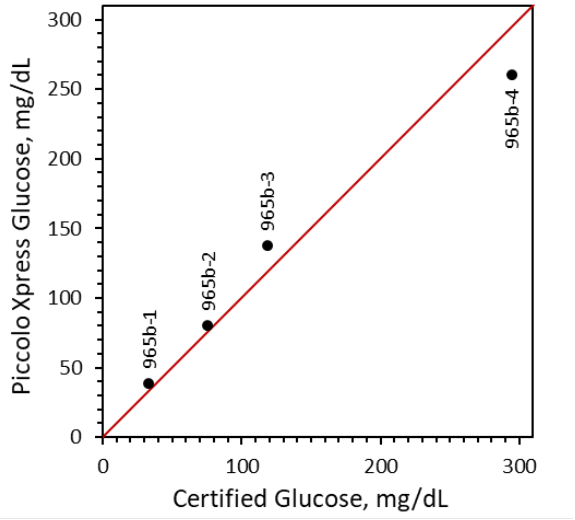

Fig. 4. Abaxis Analyzer Glucose Results for SRM 965b as a Function of Certified Values.

Each symbol represents the Piccolo Xpress Chemistry Analyzer glucose result for one level of SRM 965b as a function of the certified values. The diagonal line represents equality between the two sets of values.

The glucose results for SRM 965c provided by the Abaxis analyzer are compared to the results provided by the contractor using an AU480 Chemical Analyzer (Beckman-Coulter) in Fig. 5. All the AU480 glucose results are slightly lower than the values measured by the Abaxis analyzer. While these routine autoanalyzer platforms may be biased relative to each other and Reference Measurement Procedures, the values provided by the Abaxis and Beckman analyzers indicate SRM 965c glucose levels are within or very close to the concentration ranges requested in Statement of Work, Section 2.1.2.

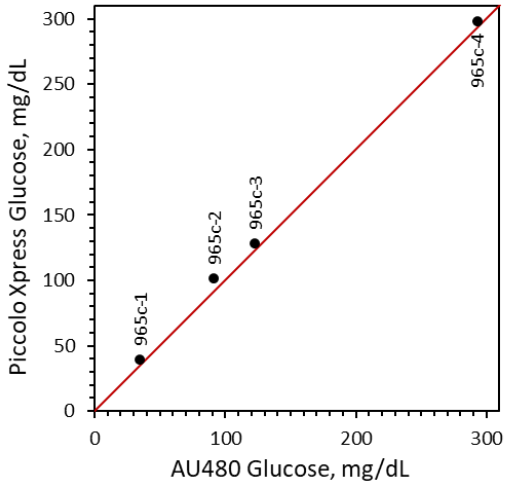

Fig. 5. Abaxis Analyzer Glucose Results for SRM 965c as a Function of Contractor's Values.

Each symbol represents the Piccolo Xpress Chemistry Analyzer glucose result for one level of SRM 965c as a function of the contractor's glucose concentration as provided by an AU480 Chemistry Analyzer. The diagonal line represents equality between the two sets of values.

3. Serum Density Determinations

Density values are needed to interconvert mass and amount-of-substance concentrations of glucose in the SRM 965c materials. Density values for the four levels of SRM 965c at the reference temperature of 23 °C were determined from gravimetric mass measurements using the Lang-Levy pipet method [10] with sample volumes of approximately 500 µL.

3.1. Materials

Three ampoules each of the four SRM 965c levels were selected for analysis. For each level, the ampoules were taken from boxes 4, 26, and 67 to representatively span their respective production lots. HPLC grade water for calibration and pipet rinsing was obtained from JT Baker. Ethanol (200 proof) for rinsing pipets was obtained from Warner-Graham.

3.2. Analysis

A 500 µL Lang-Levy pipet was calibrated with HPLC grade water that had been equilibrated for at least 24 h at ambient conditions in the same room where the balance was located. 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 pipette 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 500 µL mark with water, wiped with a lint-free cloth, and weighed. The same pipet was then cleaned and dried by attaching it to a vacuum trap, rinsing with several mL of water, 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 density of the serum at the room temperature during the measurement process, *T,* is:

$$\rho_{T\mathrm{serum}} = \frac{m_{\mathrm{serum}}}{V_{T\mathrm{serum}}} \tag{1}$$

where m_{serum} is the mass of serum that fills the pipet and $V_{T\mathrm{serum}}$ is the volume of that serum.

Since the pipet volume is fixed, the serum volume, $V_{T\mathrm{serum}}$, is equal to the water volume, $V_{T\mathrm{water}}$:

$$\rho_{T\mathrm{serum}} = \frac{m_{\mathrm{serum}}}{V_{T\mathrm{water}}} \tag{2}$$

The volumetric expansion formula for the reference temperature of 23 °C is:

$$V_{23(\mathrm{water\ or\ serum})} = V_{T(\mathrm{water\ or\ serum})}(1 - (T - 23) \cdot F) \tag{3}$$

where *T* is the room temperature at the time of measurement. Therefore:

$$\rho_{23\mathrm{serum}} = \frac{m_{\mathrm{serum}}}{V_{23\mathrm{water}}} \cdot (1 - (T - 23) \cdot F). \tag{4}$$

Given that mass does not change with temperature, $V_{23\mathrm{water}}$ is equal to the mass of water that fills the pipet, m_{water}, divided by the density of water at 23 °C, $\rho_{23\mathrm{water}}$:

$$V_{23\text{water}} = \frac{m_{\text{water}}}{\rho_{23\text{water}}}. \quad (5)$$

Therefore:

$$\rho_{23\text{serum}} = \frac{m_{\text{serum}}}{m_{\text{water}}} \cdot \rho_{23\text{water}} \cdot (1 - (T - 23) \cdot F). \quad (6)$$

The standard uncertainty for a single weighing is estimated as 0.000005 g. Since two weighings are required for each mass difference determination, the standard uncertainties for m_{water} and m_{serum}, $u(m_{\text{water}})$ and $u(m_{\text{serum}})$, are estimated as $\sqrt{2 \cdot 0.000005^2} = 0.0000071 \cong 0.00001$ g.

The temperature of the balance room is recorded at the start (T_{start}) and end (T_{end}) of three replicate determinations. The standard uncertainties of these readings are estimated as 0.005 °C. The temperature during the middle determination, T_{middle}, is estimated as (T_{start} + T_{end})/2 and is assigned a standard uncertainty 0.02 °C.

The empirically determined value for F is 0.00021 [10] and has an assigned standard uncertainty, $u(F)$, of 0.000005.

The known value for $\rho_{23\text{water}}$ is 0.99756 g/mL and is associated with a standard uncertainty, $u(\rho_{23\text{water}})$, of 0.00005 g/mL.

There is no uncertainty in the 23 °C reference temperature nor in the constant 1.

The first order (Gauss's Law) uncertainty equation for $u(\rho_{23\text{serum}})$ is [11]:

$$u(\rho_{23\text{serum}}) = \sqrt{\begin{array}{c}\left(\rho_{23\text{water}} \cdot \frac{m_{\text{serum}}}{m_{\text{water}}} \cdot F \cdot u(T)\right)^2 + \\ \left(\rho_{23\text{water}} \cdot \frac{m_{\text{serum}}}{m_{\text{water}}} \cdot (T - 23) \cdot u(F)\right)^2 + \\ (\rho_{23\text{serum}})^2 \cdot \left(\left(\frac{u(\rho_{23\text{water}})}{\rho_{23\text{water}}}\right)^2 + \left(\frac{u(m_{\text{water}})}{m_{\text{water}}}\right)^2 + \left(\frac{u(m_{\text{serum}})}{m_{\text{serum}}}\right)^2\right)\end{array}}. \quad (7)$$

3.3. SRM 965c Level 1

The three ampoules of SRM 965c Level 1 were removed from -80 °C storage and left undisturbed on the bench top to thaw at room temperature for 1.5 h each. The contents of the three ampoules were combined into a 4 mL glass vial.

The balance room temperature was 19.67 °C at the start of measurements and was 19.81 °C at the end, approximately 1.5 h later. Table 2 lists the temperatures, measured masses, mass differences, estimated serum densities at the reference temperature, and summary statistics for the set of three determinations.

Table 2. Mass Measurements for the Density Determination of SRM 965c Level 1.

	°C		g			g			g/mL	
Level 1	T	$u(T)$	m_{empty}	m_{full}	m_{water}	m_{empty}	m_{full}	m_{serum}	$\rho_{23serum}$	$u(\rho_{23serum})$
Start:	19.670	0.005	8.38894	8.89644	0.507500	8.38896	8.90940	0.52044	1.023711	0.000061
Middle:	19.740	0.020	8.38897	8.89660	0.507630	8.38900	8.90967	0.52067	1.023886	0.000061
End:	19.810	0.005	8.38895	8.89658	0.507630	8.38900	8.90961	0.52061	1.023753	0.000061

The three replicate values are readily combined with the DerSimonian-Laird uncertainty-weighed mean as implemented in the NIST Consensus Builder (NICOB) [12]. The consensus density of the SRM 965c Level 1 material is 1.02378 g/mL with a standard uncertainty of 0.00005 g/mL. The 95 % level of confidence expanded uncertainty is one-half of the coverage interval: (1.02389-1.02368)/2 = 0.00011 g/mL.

Fig. 6. Consensus Density Estimate for SRM 965c Level 1.

3.4. SRM 965c Level 2

The three ampoules of SRM 965c Level 2 were removed from -80 °C storage and left undisturbed on the bench top to thaw at room temperature for 1.5 h each. The contents of the three ampoules were combined into a 4 mL glass vial.

The balance room temperature was 19.89 °C at the start of measurements and was 19.92 °C at the end, approximately 1.5 h later. Table 3 lists the temperatures, measured masses, mass differences, estimated serum densities at the reference temperature, and summary statistics for the set of three determinations.

Table 3. Mass Measurements for the Density Determination of SRM 965c Level 2.

	°C		g			g			g/mL	
Level 2	T	$u(T)$	m_{empty}	m_{full}	m_{water}	m_{empty}	m_{full}	m_{serum}	$\rho_{23serum}$	$u(\rho_{23serum})$
Start:	19.890	0.005	8.38893	8.89649	0.50756	8.38901	8.90956	0.52055	1.023376	0.000061
Middle:	19.905	0.020	8.38899	8.89648	0.50749	8.38896	8.90928	0.52032	1.023162	0.000061
End:	19.920	0.005	8.38901	8.89638	0.50737	8.38899	8.90955	0.52056	1.023711	0.000061

The three replicate values are readily combined with the DerSimonian-Laird uncertainty-weighed mean as implemented in the NIST Consensus Builder (NICOB) [12]. The consensus density of the SRM 965c Level 2 material is 1.02342 g/mL with a standard uncertainty of 0.00016 g/mL. The 95 % level of confidence expanded uncertainty is one-half of the coverage interval: (1.02373-1.02310)/2 = 0.00032 g/mL.

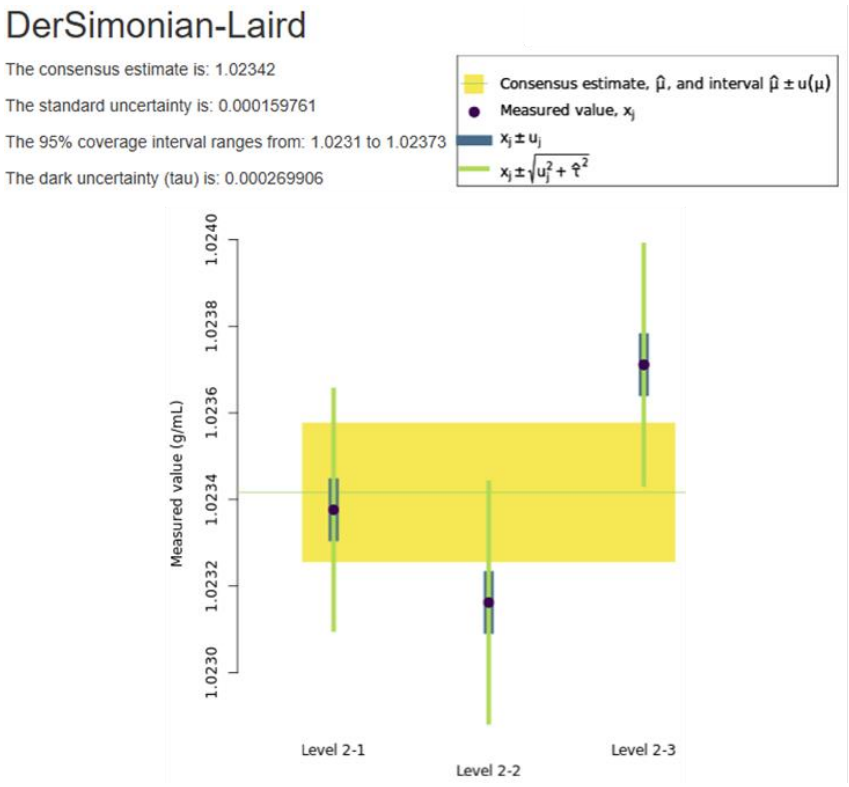

Fig. 7. Consensus Density Estimate for SRM 965c Level 2.

3.5. SRM 965c Level 3

The three ampoules of SRM 965c Level 3 were removed from -80 °C storage and left undisturbed on the bench top to thaw at room temperature for 1.5 h each. The contents of the three ampoules were combined into a 4 mL glass vial.

The balance room temperature was 19.54 °C at the start of measurements and was 19.75 °C at the end, approximately 1.5 h later. Table 4 lists the measured masses, estimated mass differences, and summary statistics for the triplicate calibration and serum mass determinations.

Table 4. Mass Measurements for the Density Determination of SRM 965c Level 3.

	°C		g			g			g/mL	
Level 3	T	$u(T)$	m_{empty}	m_{full}	m_{water}	m_{empty}	m_{full}	m_{serum}	$\rho_{23serum}$	$u(\rho_{23serum})$
Start:	19.540	0.005	8.38901	8.89664	0.50763	8.38896	8.90939	0.52043	1.023457	0.000061
Middle:	19.645	0.020	8.38896	8.89646	0.50750	8.38901	8.90952	0.52051	1.023854	0.000061
End:	19.750	0.005	8.38888	8.89643	0.50755	8.38897	8.90941	0.52044	1.023593	0.000061

The three replicate values are readily combined with the DerSimonian-Laird uncertainty-weighed mean as implemented in the NIST Consensus Builder (NICOB) [12]. The consensus density of the SRM 965c Level 3 material is 1.02363 g/mL with a standard uncertainty of 0.00012 g/mL. The 95 % level of confidence expanded uncertainty is one-half of the coverage interval: (1.02341-1.02386)/2 = 0.00023 g/mL.

Fig. 8. Consensus Density Estimate for SRM 965c Level 3.

3.6. SRM 965c Level 4

The three ampoules of SRM 965c Level 4 were removed from -80 °C storage and left undisturbed on the bench top to thaw at room temperature for 1.5 h each. The contents of the three ampoules were combined into a 4 mL glass vial.

The balance room temperature was 19.84 °C at the start of measurements and was 19.91 °C at the end, approximately 1.5 h later. Table 5 lists the measured masses, estimated mass differences, and summary statistics for the triplicate calibration and serum mass determinations.

Table 5. Mass Measurements for the Density Determination of SRM 965c Level 4.

	°C		g			g			g/mL	
Level 4	T	$u(T)$	m_{empty}	m_{full}	m_{water}	m_{empty}	m_{full}	m_{serum}	$\rho_{23serum}$	$u(\rho_{23serum})$
Start:	19.840	0.005	8.38894	8.89662	0.50768	8.38899	8.91010	0.52111	1.024629	0.000061
Middle:	19.875	0.020	8.38900	8.89672	0.50772	8.38895	8.90998	0.52103	1.024383	0.000061
End:	19.910	0.005	8.38899	8.89663	0.50764	8.38899	8.90992	0.52093	1.024340	0.000061

The three replicate values are readily combined with the DerSimonian-Laird uncertainty-weighed mean as implemented in the NIST Consensus Builder (NICOB) [12]. The consensus density of the SRM 965c Level 4 material is 1.02445 g/mL with a standard uncertainty of 0.00009 g/mL. The 95 % level of confidence expanded uncertainty is one-half of the coverage interval: (1.02463-1.02427)/2 = 0.00018 g/mL.

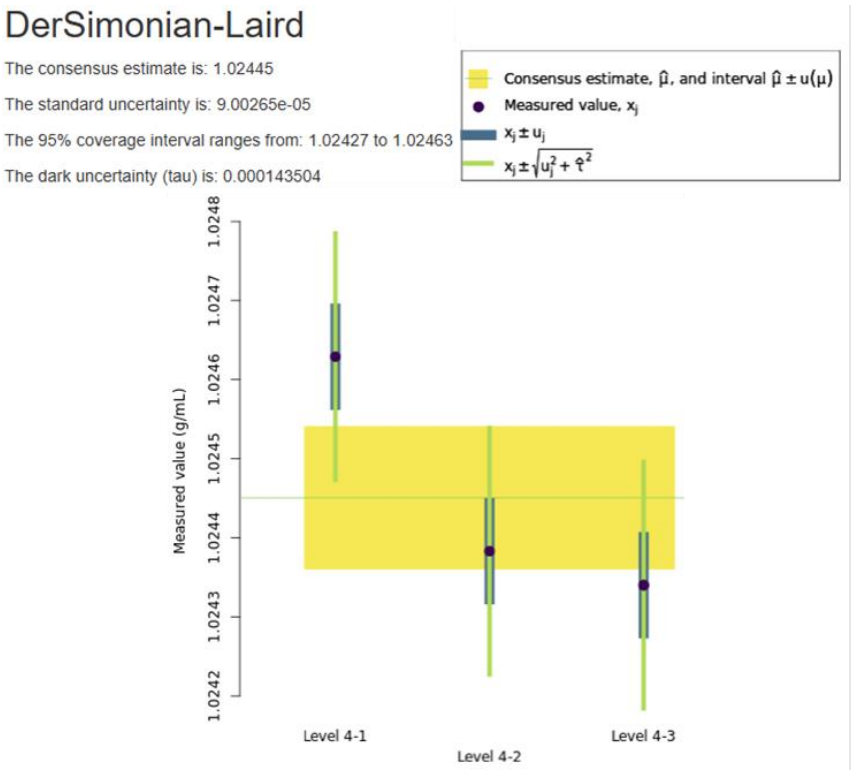

Fig. 9. Consensus Density Estimate for SRM 965c Level 4.

3.7. Dark Uncertainty

The "dark uncertainty [13]" (tau, τ) values provided with the DerSimonian-Laird analysis estimate the variability of the measurement process that is not explained by the uncertainty estimates assigned to the input parameters. Table 6 lists the estimated τ values for the four Levels of SRM 965c and their 0.00018 g/mL pooled expectation, a value three-fold larger than the 0.00006 g/mL limiting uncertainty predicted by the input parameter uncertainties.

Table 6. Unexplained Variability ("Dark Uncertainty") in Lang-Levy Measurement Process.

Material	τ, g/mL
SRM 965c Level 1	0.000068
SRM 965c Level 2	0.000270
SRM 965c Level 3	0.000192
SRM 965c Level 4	0.000143
τ_{pooled}	0.000184

Given that the 0.00001 g uncertainty assigned to the mass differences is based upon balance readability, the realizable uncertainty in the mass measurement process may be underestimated. Increasing the assigned mass measurement uncertainty to 0.00006 g raises the limiting uncertainty to 0.00018 g, essentially eliminating the dark uncertainty in the consensus estimates.

3.8. Metrological Traceability

The serum density results for the four levels of SRM 965c are metrologically traceable to the International System of Units (SI) through the calibration of the balance and thermometer used, and the established reference densities of water and the volumetric expansion formula provided in [10].

4. Glucose, Fructose, and Mannose Molecular Weight

Transforming results expressed as mass concentration to amount-of-substance concentration requires an accurate estimate of the molecular weight (molar mass) of the analyte. The molecular formula for glucose, fructose, and mannose is $C_6H_{12}O_6$. Using the IUPAC Molecular Weight calculator [14,15], the molecular weight of these monosaccharides is (180.156 ± 0.004) g/mol. The associated 95 % level of confidence interval is (180.149 to 180.163) g/mol. The calculator combines elemental atomic weights assuming that the weights are normally distributed across their stated uncertainty intervals; Fig. 10 displays the resulting probability density for $C_6H_{12}O_6$.

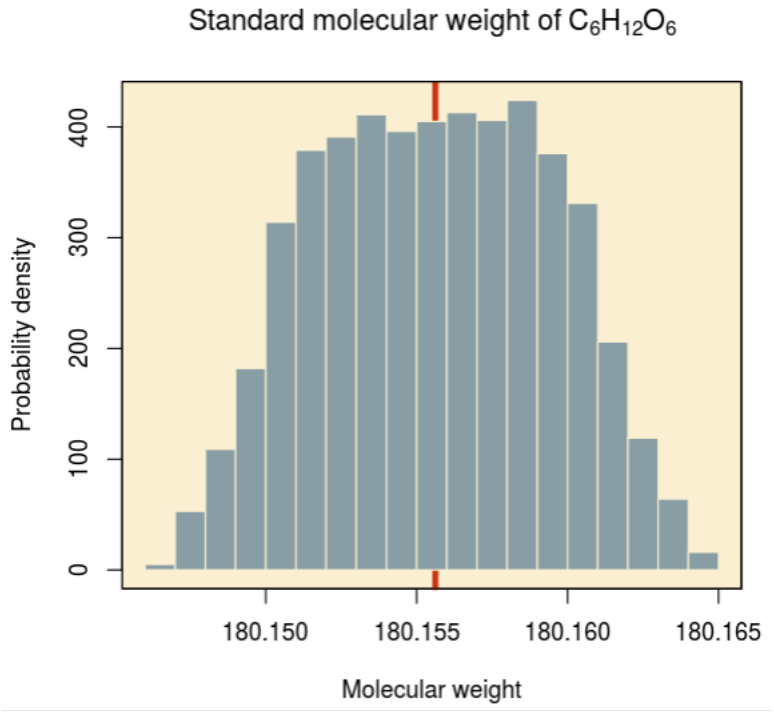

Fig. 10. Probability Density for the Molecular Weight of Glucose.

5. NIST ID-LC-MS/MS Measurements

The glucose concentrations in the previous members of the SRM 965 series were assigned using the NIST's JCTLM-recognized ID-GC-MS RMP [16]. This method involves a complicated, time-consuming two-step derivatization process with overnight equilibration as well as use of hazardous chemicals. The development of ID-LC-MS/MS RMPs for serum glucose provide high-accuracy results with simpler and quicker sample preparation. The NIST implementation of the JCTLM-recognized RMP developed by Zhang et al. [6] has been validated with SRM 965b.

Samples were processed and run in six batches ("days") over a two-week interval. Each day included three replicates of three blanks, three replicates of three calibrants, one SRM 965b sample of two of its four Levels, and three or four SRM 965c samples of each its four levels. For each day at each level, three of the SRM 965c samples were each from a different ampoule. When included, a fourth SRM 965c sample was a replicate of one of the three ampoules. Each day involved 33 or 34 analyses. The analysis order was designed to enable evaluating between-vial heterogeneity and between-day analytical statistical control.

The following sections describe the sample preparation and instrumental methods used to quantitatively characterize the samples.

5.1. Stock Preparation

A mass fraction of 0.1 % aqueous sodium azide solution was made up using Milli-Q water (minimum resistivity 18.2 MΩ-cm). This solution was used for preparing labeled and unlabeled glucose stocks. An ≈1 mg/g labeled internal standard (IS) glucose solution was prepared by accurately weighing 20 mg of D-Glucose-$^{13}C_6$ (Sigma-Aldrich, ≥ 99.0 %) and 20 mL of the sodium azide solution into a 50 mL centrifuge tube. Two sets of ≈1 mg/g unlabeled glucose solutions were gravimetrically prepared by combining 20 mg of SRM 917d [9] and 20 mL of the sodium azide solution into 50 mL plastic centrifuge tubes. Table 1 lists the stock solutions and the relevant masses.

Table 7. Stock Solutions.

Stock	m_{917d}, g	m_{IS}, g	m_{Stock}, g	w_{Stock}, mg/g
917d-1	0.02445		20.12840	1.2098 [a]
917d-2	0.02248		20.11060	1.1133 [a]
917d-3	0.02139		20.01397	1.0645 [a]
917d-4	0.02698		20.03834	1.3410 [a]
IS-1		0.01985	20.13725	0.9818 [b]
IS-2		0.02037	20.06670	1.0111 [b]

a Concentration of unlabeled glucose in the SRM 917d stock solutions, using the certified purity: *P*= 996 mg/g.
b Concentration of ^{13}C glucose in the Spike stock solutions, using a nominal purity: *P* = 1000 mg/g.

The mass fraction glucose concentrations of these stocks are calculated:

$$w_{\mathrm{Stock}} = P\frac{m_{917\mathrm{d}}\ \mathrm{or}\ m_{\mathrm{IS}}}{m_{\mathrm{Stock}}} \quad (8)$$

where *P* is the certified (SRM 917d) or nominal (IS Spike) purity of the calibration materials. The concentrations of the $^{13}C_6$ IS in the Spike stocks are not required for quantitation, but approximate values are required for efficient sample preparation.

A 1-phenyl-3-methyl-5-pyrazalone (PMP, Spectrum Chemical) derivatization solution was made fresh each week by diluting 2.18 g of PMP in 50 mL of 400 mmol/L ammonium hydroxide solution. The ammonium hydroxide solution was prepared by diluting 1.35 mL of ammonium hydroxide in 50 mL of Milli-Q water.

5.2. Sample and Calibrant Preparation

All ampoules of frozen serum to be analyzed in a given day were removed from the freezer at the same time and allowed to warm to room temperature for approximately 30 minutes prior to weighing. Samples were mixed by gently swirling, and 250 µL was sampled with a pipette and weighed into plastic Eppendorf tubes containing 90 µL to 350 µL aliquots of the labeled glucose solution to give approximate 1:1 mass ratio of unlabeled:labeled glucose. Upon completion of weighing, all samples were allowed to equilibrate at room temperature for one hour.

Three glucose standards were gravimetrically prepared in Eppendorf tubes with 200 µL of the ^{13}C labeled IS solution and 0.25 mL, 0.2 mL, or 0.15 mL of a 917d stock solution. The mass ratios of unlabeled to labeled glucose ranged from 0.8 to 1.2. Calibration standards were derivatized alongside samples.

All samples and calibrants were deproteinized and diluted by adding 1.5 mL of ice-cold absolute ethanol to each tube, quickly vortexed, and placed in a freezer at -20 °C for 15 minutes. Samples were then centrifuged for 10 minutes at 4 °C and 1,466 rad/s (14 000 rpm). Using a pipet, 25 µL of the supernatant was transferred into 10 mL glass tubes and combined with 25 µL of PMP derivatization solution. Samples were placed in an oven at 70 °C for 30 minutes. Upon removal from the oven, 50 µL of 1 mol/L acetic acid prepared in milli-Q water was added to each sample. Each sample was then diluted with 1 mL of milli-Q water.

Excess PMP was removed by twice washing the mixture with chloroform. The first wash added 1 mL chloroform to each sample which was quickly vortexed followed by removing the bottom chloroform layer from the tube using a glass pipet. The second wash added 1 mL of chloroform to each tube, the sample was vortexed, and 100 µL of the top layer was transferred to an autosampler vial. Each sample was diluted with 900 µL of milli-Q water for analysis.

5.3. Instrumental Methods

Two LC-MS/MS separation and detection methods were used to characterize split-aliquots of the prepared samples. Results to be used in glucose concentration certification were made using a method adapted from the RMP described in [6]. A second set of results were obtained on aliquots of the same samples using a quite different form of mass spectrometric detection. These results were intended to help characterize the method and, if successful, to confirm the reliability of the certification results.

5.3.1. Reference Measurement Procedure

Samples were analyzed using an Agilent 1260 Infinity II LC coupled to a Sciex Triple Quad 5500+ mass spectrometer.

The HPLC separation was performed on a ZORBAX 300Extend C_{18} column (3.5 µm, 2.1 mm × 150 mm, Agilent) with a mobile phase consisting of 0.05 % acetic acid in water–acetonitrile (75:25, volume ratio) at a flow rate of 0.3 mL/min.

MS detection was operated in positive electrospray ionization (ESI) mode with multiple reaction monitoring (MRM). Nitrogen was used as the curtain gas (CUR) at 138 kPa (20 psi), nebulizer gas (GS1) at 413 kPa (60 psi), auxiliary gas (GS2) at 448 kPa (65 psi), and collision gas (CAD) at 48 kPa (7 psi). The electrospray voltage was set to 5500 V, and the turbo gas temperature (TEM) was maintained at 700 °C. The dwell times were 0.15 s. The injection volume was 5 µL.

The transitions at *m*/*z* 511.2→373.2 for glucose and *m*/*z* 517.2→375.2 for labeled glucose were monitored, respectively. The ions pairs of *m*/*z* 511.2→175.1 for glucose and m/z 517.2→175.1 for labeled glucose were monitored for confirmation. The declustering potential (DP) was 201 V for the quantification ions and 176 V for the confirmation ions. The entrance potential (EP) was 10 V for both ions, the collision energy (CE) was 37 eV for both ions, and the collision exit potential (CXP) was 8 V for the quantification ions and 12 V for the confirmation ions.

Skyline (version 23.1.0.380) was used to integrate selected peaks.

5.3.2. Orbitrap Method

Samples were analyzed using a Vanquish UHPLC coupled to a Q Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA).

The HPLC separation was performed on a ZORBAX 300Extend C_{18} column (3.5 µm, 2.1 mm × 150 mm, Agilent) at 35 °C. The injection volume was 5 µL. The flow rate was 0.3 mL/min. Mobile phase A was 0.05 % acetic acid in water. Mobile phase B was 0.05 % acetic acid in acetonitrile. The gradient program was a linear ramp from 95 % A to 75 % A at 10 min, hold for 2 min, linear ramp back to 95 % A over 0.5 min, hold for 4.5 min.

MS detection was operated in positive electrospray ionization (ESI) mode with parallel reaction monitoring (PRM). The MS1 inclusion list ions were *m*/*z* 511.2 glucose and *m*/*z* 517.2 labeled glucose. Precursor fragmentation was performed using a collision energy of 35 and quadrupole isolation at *m*/*z* 1.0 width. The fragment scan resolution using the orbitrap was set at 35k. The fragment ion target value was 2.0×10^5 and a maximum injection time of 100 ms.

Skyline (version 23.1.0.380) was used to integrate selected peaks and to obtain a combined peak area that included the following transitions: *m*/*z* 511.2 → {373.16, 241.1, 217.09, 187.09, 175.09} for glucose and *m*/*z* 517.2 → {375.17, 245.11, 219.1, 188.09, 175.09} for labeled glucose.

5.4. Quantitation

Mass fraction serum glucose results for the SRM 965b and 965c materials were calculated using two implementations of an internal standard approach. Both approaches use response factors (RFs) calculated for each injection of a calibrant:

$$R = \frac{A_{917}}{A_{\mathrm{IS}}} \frac{m_{\mathrm{IS}}}{w_{917} \cdot m_{917}} \tag{9}$$

where R is the RF, A_{917} is the area of SRM 917d glucose peak(s), A_{IS} is the area of labeled glucose peak(s), m_{IS} is the mass of the IS solution, $w_{917\mathrm{d}}$ is the mass fraction of glucose in the SRM 917d stock solution, and m_{917} is the mass of that solution.

A representative response factor determined from multiple injections of multiple calibrants is used to calculate the mass fraction of glucose in each of the SRM 965b or 965c samples, w_{965}:

$$w_{965} = \left(\frac{A_{965}}{A_{\mathrm{IS}}}\right) \left(\frac{m_{\mathrm{IS}}}{m_{965}}\right) / R \tag{10}$$

where A_{965} is the area of SRM 965b or 965c glucose peak(s) and m_{965} is the mass of the sample.

5.4.1. Daily Mean Response Factors

Three calibrants were included in each of the six sets of analysis. Each calibrant was injected in triplicate. The least complex estimate of R for each calibrant on a given day is calculated from the mean of the three A_{917}/A_{IS} ratios:

$$R_{\mathrm{Day}} = \frac{m_{\mathrm{IS}}}{w_{917} \cdot m_{917}} \left(\sum_{i=1}^{3} \frac{A_{917,i}}{A_{\mathrm{IS},i}}\right) / 3 \tag{11}$$

where i indexes over the injections. Likewise, the least complex estimate of R for a given day is calculated from the mean of the three calibrants:

$$\bar{R}_{\mathrm{Day}} = \left(\sum_{j=1}^{3} R_{\mathrm{Day},j}\right) / 3 \tag{12}$$

where j indexes over the calibrants.

The masses, peak areas, peak area ratios, and daily mean response factors (DMRFs) for Days 1, 2, and 3 are listed in Table 8; the values for Days 4, 5, and 6 are listed in Table 9. The masses, peak areas, peak area ratios, DMRF-estimated serum glucose concentrations, and summary statistics for the four SRM 965b Levels are listed in Table 10; the values for the four SRM 965c Levels are listed in Table 11, Table 12, Table 13, and Table 14.

Table 8. Calibration Data and Mean Response Factors for Days 1, 2, and 3.

		Calibrant Composition				Reference Measurement Procedure				Orbitrap Detection			
		Stocks		Masses		Peak Areas		A_{917}/A_{IS}	R_{Day}	$\bar{R}_{Day}$	Peak Areas		A_{917}/A_{IS}	R_{Day}	$\bar{R}_{Day}$
Day	Calibrant	w_{917}, mg/g	IS	m_{917}, g	m_{IS}, g	A_{917}	A_{IS}				A_{917}	A_{IS}			
1	Cal01a	1.2098 (917d-1)	IS-1	0.24933	0.20020	15351797	9164164	1.6752		1.1236	333575488	213597472	1.5617		1.0505
	Cal01b					12499294	7523218	1.6614	1.1053		275170272	176828624	1.5561	1.0376	
	Cal01c					12595943	7591256	1.6593			233399584	148448848	1.5723		
	Cal02a	1.1133 (917d-2)	IS-1	0.20032	0.20116	9676545	7460616	1.2970			259304880	206368800	1.2565		
	Cal02b					8872451	6874695	1.2906	1.1668		199494880	171197408	1.1653	1.0898	
	Cal02c					8221216	6357902	1.2931			162393552	135001648	1.2029		
	Cal03a	1.2098 (917d-1)	IS-1	0.14971	0.20061	10426915	10491001	0.9939			235967712	247000160	0.9553		
	Cal03b					9429469	9476836	0.9950	1.0988		186451072	206568576	0.9026	1.0241	
	Cal03c					8733664	8846579	0.9872			159582144	174259424	0.9158		
2	Cal04a	1.1133 (917d-2)	IS-1	0.24978	0.19847	13286768	8196981	1.6209		1.1378	297213600	186369088	1.5948		1.0970
	Cal04b					12422164	7629228	1.6282	1.1583		255418704	163245984	1.5646	1.1149	
	Cal04c					11460789	7076276	1.6196			205927152	134867904	1.5269		
	Cal05a	1.2098 (917d-1)	IS-1	0.20056	0.19818	8098623	5992803	1.3514			198373008	147737776	1.3427		
	Cal05b					7319109	5396140	1.3564	1.1047		162706416	125504168	1.2964	1.0660	
	Cal05c					6893589	5107182	1.3498			136171456	106707024	1.2761		
	Cal06a	1.1133 (917d-2)	IS-1	0.15078	0.19822	9771900	9987074	0.9785			218644864	233751328	0.9354		
	Cal06b					8650625	8852864	0.9772	1.1504		192565696	200502800	0.9604	1.1102	
	Cal06c					7912688	8183050	0.9670			158551136	171433632	0.9249		
	Cal07a	1.2098 (917d-1)	IS-1	0.25005	0.20055	23160270	14016071	1.6524		1.1193	582173568	366244736	1.5896		1.0454
	Cal07b					22220678	13319208	1.6683	1.0994		494453728	318402432	1.5529	1.0354	
	Cal07c					20629324	12469578	1.6544			436469024	282899968	1.5428		
	Cal08a	1.1133 (917d-2)	IS-1	0.20164	0.20111	19696910	15195640	1.2962			537212864	431838368	1.2440		
	Cal08b					18456968	14170917	1.3025	1.1615		443790784	366575424	1.2106	1.0817	
	Cal08c					17319438	13415578	1.2910			376712064	322582592	1.1678		
	Cal09a	1.2098 (917d-1)	IS-1	0.14888	0.20024	10626605	10782330	0.9856			275111936	287632960	0.9565		
	Cal09b					9567861	9702110	0.9862	1.0969		220020720	245343520	0.8968	1.0191	
	Cal09c					9001884	9107980	0.9884			177455312	197857120	0.8969		

Table 9. Calibration Data and Mean Response Factors for Days 4, 5, and 6.

		Calibrant Composition				Reference Measurement Procedure					Orbitrap Detection				
		Stocks		Masses		Peak Areas		A_{917}/A_{IS}		R_{Day}	Peak Areas		A_{917}/A_{IS}		R_{Day}
Day	Calibrant	w_{917}, mg/g	IS	m_{917}, g	m_{IS}, g	A_{917}	A_{IS}	Ratio	Mean		A_{917}	A_{IS}	Ratio	Mean	
4	Cal10a	1.3410 (917d-4)	IS-2	0.2503	0.19924	4771032	2528070	1.8872		1.1174	202823648	108465528	1.8699		1.0844
	Cal10b					4261888	2264785	1.8818	1.1228		172314176	93605792	1.8409	1.0931	
	Cal10c					3966969	2081735	1.9056			150987376	83250920	1.8136		
	Cal11a	1.0645 (917d-3)	IS-2	0.20072	0.19932	4054837	3421231	1.1852			190195968	163193328	1.1655		
	Cal11b					3779646	3211654	1.1769	1.1039		168487168	149388432	1.1279	1.0651	
	Cal11c					3486599	2934985	1.1879			138411312	122278592	1.1319		
	Cal12a	1.3410 (917d-4)	IS-2	0.14922	0.20024	7430267	6599160	1.1259			297103456	263357824	1.1281		
	Cal12b					6732150	5982766	1.1253	1.1256		263894560	244206912	1.0806	1.0951	
	Cal12c					6398801	5695682	1.1234			231222592	215203680	1.0744		
5	Cal13a	1.0645 (917d-3)	IS-2	0.24957	0.19987	4332191	2921913	1.4827		1.1199	196689328	138943968	1.4156		1.0548
	Cal13b					3925455	2652029	1.4802	1.1178		171622752	123653072	1.3879	1.0492	
	Cal13c					3698222	2474504	1.4945			141516224	102515648	1.3804		
	Cal14a	1.3410 (917d-4)	IS-2	0.20113	0.20074	7377350	4918781	1.4998			341319648	234731408	1.4541		
	Cal14b					6692608	4431905	1.5101	1.1197		284490944	202252704	1.4066	1.0602	
	Cal14c					6320117	4203780	1.5034			246482192	174466928	1.4128		
	Cal15a	1.0645 (917d-3)	IS-2	0.15075	0.20038	4031927	4456149	0.9048			174192160	200762208	0.8677		
	Cal15b					3670578	4082213	0.8992	1.1223		145712128	175401792	0.8307	1.0551	
	Cal15c					3403116	3813783	0.8923			120389344	143899632	0.8366		
6	Cal16a	1.3410 (917d-4)	IS-2	0.20962	0.19964	8138993	5145863	1.5817		1.1240	413200480	275195520	1.5015		1.0538
	Cal16b					7420710	4697452	1.5797	1.1212		366486400	245616128	1.4921	1.0529	
	Cal16c					6791991	4313614	1.5745			313677312	215756576	1.4539		
	Cal17a	1.0645 (917d-3)	IS-2	0.20063	0.2005	6897426	5712347	1.2075			332294144	288153280	1.1532		
	Cal17b					6034531	5006068	1.2054	1.1309		288911424	256914848	1.1245	1.0639	
	Cal17c					5516742	4593619	1.2010			238743184	212755600	1.1222		
	Cal18a	1.3410 (917d-4)	IS-2	0.13935	0.20033	5117927	4875300	1.0498			257995936	259673584	0.9935		
	Cal18b					4408992	4213952	1.0463	1.1200		216055712	221344864	0.9761	1.0445	
	Cal18c					4101545	3950762	1.0382			184579136	193613248	0.9533		

Table 10. SRM 965b Levels 1 to 4: Sample Composition, Peak Areas, and Glucose Concentration.

			Sample Masses		Reference Measurement Procedure					Orbitrap Detection				
Level	Day	Sample	m_{965}, g	m_{IS}, g	A_{965}	A_{IS}	A_{965}/A_{IS}	R_{Day}	w_{965}, mg/g	A_{965}	A_{IS}	A_{965}/A_{IS}	RF_{Day}	w_{965}, mg/g
1	2	965b-L1-01	0.24401	0.08911	3501329	3535688	0.99028	1.1378	0.3178	70757056	73266320	0.96575	1.0970	0.3215
	5	965b-L1-02	0.24981	0.08993	5442514	5368540	1.01378	1.1199	0.3259	77893000	82439680	0.94485	1.0548	0.3225
	6	965b-L1-03	0.24977	0.08905	2554490	2517671	1.01462	1.1240	0.3218	133411312	138137536	0.96579	1.0538	0.3268
								Mean:	0.3218				Mean:	0.3236
								Standard Deviation:	0.0040				Standard Deviation:	0.0028
								Relative Standard Deviation (%):	1.2				Relative Standard Deviation (%):	0.9
2	1	965b-L2-01	0.24406	0.19916	5957972	5758535	1.03463	1.1236	0.7514	114908976	120852160	0.95082	1.0505	0.7386
	5	965b-L2-04	0.24592	0.20028	1955603	1910629	1.02354	1.1199	0.7443	217108064	229359456	0.94658	1.0548	0.7308
	6	965b-L2-03	0.24479	0.20036	6201971	6083577	1.01946	1.1240	0.7423	312235584	327483200	0.95344	1.0538	0.7406
								Mean:	0.7460				Mean:	0.7367
								Standard Deviation:	0.0048				Standard Deviation:	0.0051
								Relative Standard Deviation (%):	0.6				Relative Standard Deviation (%):	0.7
3	2	965b-L3-01	0.24285	0.29779	13998786	13432301	1.04217	1.1378	1.1232	310357088	304847200	1.01807	1.0970	1.1380
	4	965b-L3-02	0.24912	0.29969	5182454	4713122	1.09958	1.1174	1.1838	233395744	214445856	1.08837	1.0844	1.2074
	5	965b-L3-03	0.24555	0.30046	6092728	5896980	1.03319	1.1199	1.1288	267002320	273807232	0.97515	1.0548	1.1312
								Mean:	1.1453				Mean:	1.1588
								Standard Deviation:	0.0335				Standard Deviation:	0.0422
								Relative Standard Deviation (%):	2.9				Relative Standard Deviation (%):	3.6
4	1	965b-L4-01	0.12261	0.34991	10854979	9642169	1.12578	1.1236	2.8593	183365312	177957952	1.03039	1.0505	2.7992
	3	965b-L4-02	0.12114	0.34945	9995464	9048512	1.10465	1.1193	2.8470	235673280	226588448	1.04009	1.0454	2.8700
	4	965b-L4-03	0.12152	0.34918	4550880	4084358	1.11422	1.1174	2.8652	202608416	187211968	1.08224	1.0844	2.8676
								Mean:	2.8572				Mean:	2.8456
								Standard Deviation:	0.0093				Standard Deviation:	0.0402
								Relative Standard Deviation (%):	0.3				Relative Standard Deviation (%):	1.4

Table 11. SRM 965c Level 1: Sample Composition, Peak Areas, and Glucose Concentration.

				Sample Masses		Reference Measurement Procedure					Orbitrap Detection				
Day	Sample	Box	Rep	m_{965}, g	m_{IS}, g	A_{965}	A_{IS}	A_{965}/A_{IS}	RF_{Day}	w_{965}, mg/g	A_{965}	A_{IS}	A_{965}/A_{IS}	RF_{Day}	w_{965}, mg/g
1	965c-L1-01	1	1	0.24787	0.08915	4062703	3927113	1.0345	1.1236	0.3311	80760768	84453440	0.95628	1.0505	0.3274
	965c-L1-02	1	2	0.24602	0.08896	4682868	4580930	1.0223		0.3290	95944896	100420560	0.95543		0.3289
	965c-L1-03	64	1	0.24802	0.08881	5469763	5299701	1.0321		0.3289	106262184	107927312	0.98457		0.3356
	965c-L1-04	14	1	0.24608	0.08935	3018441	2953060	1.0221		0.3303	51593784	53893568	0.95733		0.3309
2	965c-L1-05	50	1	0.24461	0.08946	2984160	2940836	1.0147	1.1378	0.3262	61101128	62896124	0.97146	1.0970	0.3382
	965c-L1-06	28	1	0.24833	0.08925	3911456	3909700	1.0004		0.3160	82860064	84409016	0.98165		0.3358
	965c-L1-07	4	1	0.24241	0.08907	4740174	4692576	1.0101		0.3262	104235720	107191544	0.97242		0.3401
3	965c-L1-08	16	1	0.24503	0.08986	3004799	2981355	1.0079	1.1193	0.3302	65984700	71823104	0.91871	1.0454	0.3207
	965c-L1-09	39	1	0.23791	0.08988	3320615	3436159	0.9664		0.3262	76335104	84964536	0.89843		0.3231
	965c-L1-10	53	1	0.24506	0.08997	4140657	4004764	1.0339		0.3391	84914496	89065712	0.95339		0.3332
4	965c-L1-11	32	1	0.24535	0.09009	1628638	1650817	0.9866	1.1174	0.3242	75529120	79038280	0.95560	1.0844	0.3340
	965c-L1-12	59	1	0.24811	0.09032	600586	619606	0.9693		0.3158	25119884	27413860	0.91632		0.3175
	965c-L1-13	67	1	0.24160	0.08997	2399880	2428432	0.9882		0.3293	98063904	104864112	0.93515		0.3315
5	965c-L1-14	74	1	0.24592	0.08999	1947349	1922368	1.0130	1.1199	0.3310	76138424	80820792	0.94206	1.0548	0.3282
	965c-L1-15	23	1	0.24387	0.09002	3078212	3075412	1.0009		0.3299	138099200	145932208	0.94632		0.3325
	965c-L1-16	47	1	0.24557	0.09011	2073862	2060005	1.0067		0.3299	97597216	103112720	0.94651		0.3306
6	965c-L1-17	36	1	0.23863	0.08892	2107024	2155223	0.9776	1.1240	0.3241	113044336	122937200	0.91953	1.0538	0.3262
	965c-L1-18	75	1	0.23877	0.08888	2332372	2337442	0.9978		0.3304	113789288	123079752	0.92452		0.3276
	965c-L1-19	7	1	0.24719	0.08892	2579741	2551007	1.0113		0.3236	130661248	135147488	0.96680		0.3311
								Number of Samples:		19			Number of Samples:		19
								Mean:		0.3274			Mean:		0.3302
								Standard Deviation		0.0054			Standard Deviation		0.0057
								Relative Standard Deviation (%)		1.6			Relative Standard Deviation (%)		1.7

Table 12. SRM 965c Level 2: Sample Composition, Peak Areas, and Glucose Concentration.

				Sample Masses		Reference Measurement Procedure					Orbitrap Detection				
Day	Sample	Box	Rep	m_{965}, g	m_{IS}, g	A_{965}	A_{IS}	A_{965}/A_{IS}	RF_{Day}	w_{965}, mg/g	A_{965}	A_{IS}	A_{965}/A_{IS}	RF_{Day}	w_{965}, mg/g
1	965c-L2-01	4	1	0.25106	0.19908	11617151	8667930	1.3402	1.1236	0.9458	204246512	165652672	1.23298	1.0505	0.9307
	965c-L2-02	25	1	0.24863	0.19853	9373564	7095659	1.3210		0.9388	191887168	157014832	1.22210		0.9289
	965c-L2-03	48	1	0.24957	0.19932	5629912	4304485	1.3079		0.9296	104092480	86586216	1.20218		0.9140
2	965c-L2-04	16	1	0.24744	0.19885	10998315	8363979	1.3150	1.1378	0.9288	223028480	176749440	1.26183	1.0970	0.9653
	965c-L2-05	42	1	0.24812	0.19878	12126813	9222462	1.3149		0.9259	278243392	221337696	1.25710		0.9587
	965c-L2-06	42	2	0.25212	0.19922	10391471	7746688	1.3414		0.9316	229827968	179913232	1.27744		0.9609
	965c-L2-07	56	1	0.24892	0.19896	7507217	5736986	1.3086		0.9193	155664672	125947360	1.23595		0.9404
3	965c-L2-08	33	1	0.24300	0.20005	11723373	9222198	1.2712	1.1193	0.9350	447064192	354963264	1.25947	1.0454	0.9870
	965c-L2-09	59	1	0.24428	0.19993	19318178	14403190	1.3412		0.9808	274296352	230410224	1.19047		0.9275
	965c-L2-10	70	1	0.24632	0.20008	13807865	10621771	1.3000		0.9434	254252864	220379536	1.15370		0.8921
4	965c-L2-11	22	1	0.24573	0.20073	5743385	4433064	1.2956	1.1174	0.9471	277264960	225427376	1.22995	1.0844	0.9564
	965c-L2-12	46	1	0.24676	0.20106	5699538	4422657	1.2887		0.9397	314384768	257432640	1.22123		0.9472
	965c-L2-13	71	1	0.24535	0.20110	7176553	5599900	1.2816		0.9400	269330560	211762144	1.27185		0.9924
5	965c-L2-14	10	1	0.24419	0.19890	6489744	5160682	1.2575	1.1199	0.9146	255542240	195354000	1.30810	1.0548	1.0143
	965c-L2-15	37	1	0.24591	0.19894	6211307	4497827	1.3810		0.9975	334888832	277492640	1.20684		0.9294
	965c-L2-16	37	2	0.24579	0.19962	7996053	6418608	1.2458		0.9034	279220928	235396704	1.18617		0.9170
	965c-L2-17	75	1	0.24334	0.19915	7209706	5608251	1.2856		0.9394	290862080	241874336	1.20253		0.9368
6	965c-L2-18	1	1	0.24593	0.19977	6281874	4959759	1.2666	1.1240	0.9153	289690432	237050864	1.22206	1.0538	0.9450
	965c-L2-19	12	1	0.24885	0.20034	5695681	4391412	1.2970		0.9289	346525824	286403008	1.20992		0.9272
	965c-L2-20	64	1	0.24484	0.20068	6718406	5245065	1.2809		0.9340	293010368	247806016	1.18242		0.9226
									Number of Samples:	20				Number of Samples:	20
									Mean:	0.937				Mean:	0.945
									Standard Deviation	0.021				Standard Deviation	0.029
									Relative Standard Deviation (%)	2.3				Relative Standard Deviation (%)	3.1

Table 13. SRM 965c Level 3: Sample Composition, Peak Areas, and Glucose Concentration.

				Sample Masses		Reference Measurement Procedure					Orbitrap Detection				
Day	Sample	Box	Rep	m_{965}, g	m_{IS}, g	A_{965}	A_{IS}	A_{965}/A_{IS}	RF_{Day}	w_{965}, mg/g	A_{965}	A_{IS}	A_{965}/A_{IS}	RF_{Day}	w_{965}, mg/g
1	965c-L3-01	18	1	0.24461	0.29725	8264318	7306286	1.1311	1.1236	1.2233	169908304	161949488	1.04914	1.0505	1.2136
	965c-L3-02	40	1	0.25040	0.29909	7836251	6827685	1.1477		1.2201	168627840	155362112	1.08539		1.2341
	965c-L3-03	56	1	0.25100	0.29899	7000116	6106297	1.1464		1.2153	129597656	122836632	1.05504		1.1963
2	965c-L3-04	31	1	0.24817	0.29458	14462838	12696978	1.1391	1.1378	1.1883	291967584	265741536	1.09869	1.0970	1.2415
	965c-L3-05	58	1	0.24865	0.30214	14897171	13462598	1.1066		1.1818	338465536	317613856	1.06565		1.2326
	965c-L3-06	70	1	0.24922	0.29580	14389387	12810116	1.1233		1.1718	287329824	274099904	1.04827		1.1844
3	965c-L3-07	24	1	0.23924	0.29949	6888183	6484997	1.0622	1.1193	1.1880	488628992	464761696	1.05135	1.0454	1.2529
	965c-L3-08	46	1	0.24543	0.30003	21135062	19225266	1.0993		1.2007	413976224	383033088	1.08078		1.2577
	965c-L3-09	74	1	0.24596	0.30020	21424994	18649444	1.1488		1.2528	386538848	390305504	0.99035		1.1506
	965c-L3-10	74	2	0.23889	0.29942	18327352	17396040	1.0535		1.1798	178021472	178247264	0.99873		1.1916
4	965c-L3-11	10	1	0.24486	0.29991	6353733	5737284	1.1074	1.1174	1.2139	246611744	234946928	1.04965	1.0844	1.2238
	965c-L3-12	38	1	0.24644	0.30025	1001834	903526	1.1088		1.2089	243922784	225248352	1.08291		1.2559
	965c-L3-13	75	1	0.24608	0.29970	5200727	4666225	1.1145		1.2148	47198480	44200248	1.06783		1.2380
5	965c-L3-14	1	1	0.24543	0.29990	8318198	7727799	1.0764	1.1199	1.1744	301626880	298156224	1.01164	1.0548	1.1767
	965c-L3-15	14	1	0.24040	0.29920	9027076	8234410	1.0963		1.2183	320944000	302967680	1.05933		1.2551
	965c-L3-16	63	1	0.24508	0.29891	7358913	6648489	1.1069		1.2054	382929600	359709184	1.06455		1.2360
6	965c-L3-17	6	1	0.24725	0.29999	8947273	8008602	1.1172	1.1240	1.2059	444839872	442888768	1.00441	1.0538	1.1601
	965c-L3-18	28	1	0.24800	0.29950	6430390	5693204	1.1295		1.2135	446827712	417939264	1.06912		1.2291
	965c-L3-19	49	1	0.24053	0.29920	9460691	8727201	1.0840		1.1997	347731200	328105792	1.05981		1.2549
								Number of Samples:		19			Number of Samples:		19
								Mean:		1.204			Mean:		1.220
								Standard Deviation		0.020			Standard Deviation		0.034
								Relative Standard Deviation (%)		1.5			Relative Standard Deviation (%)		2.8

Table 14. SRM 965c Level 4: Sample Composition, Peak Areas, and Glucose Concentration.

				Sample Masses		Reference Measurement Procedure					Orbitrap Detection				
Day	Sample	Box	Rep	m_{965}, g	m_{IS}, g	A_{965}	A_{IS}	A_{965}/A_{IS}	RF_{Day}	w_{965}, mg/g	A_{965}	A_{IS}	A_{965}/A_{IS}	RF_{Day}	w_{965}, mg/g
1	965c-L4-01	31	1	0.11950	0.34897	13549756	11924233	1.1363	1.1236	2.9532	237922864	225423008	1.05545	1.0505	2.9340
	965c-L4-02	60	1	0.11478	0.34906	13512641	12410462	1.0888		2.9469	243359872	242846064	1.00212		2.9011
	965c-L4-03	68	1	0.12145	0.35002	14012395	12075053	1.1604		2.9764	303612480	277274624	1.09499		3.0041
2	965c-L4-04	24	1	0.12226	0.34780	8832073	7600366	1.1621	1.1378	2.9054	225296720	201075328	1.12046	1.0970	3.0342
	965c-L4-05	45	1	0.12282	0.35164	11265988	9729316	1.1579		2.9137	235999200	208163840	1.13372		3.0899
	965c-L4-06	72	1	0.12312	0.34881	10428749	9024917	1.1556		2.8773	187500400	169751936	1.10456		2.9789
3	965c-L4-07	9	1	0.12169	0.34979	19369404	17462686	1.1092	1.1193	2.8486	370101984	354398720	1.04431	1.0454	2.8575
	965c-L4-08	38	1	0.12169	0.34973	18916950	16451788	1.1498		2.9524	250299456	236888240	1.05661		2.8907
	965c-L4-09	75	1	0.12148	0.34960	11766938	10247992	1.1482		2.9523	449680832	416157312	1.08055		2.9602
4	965c-L4-10	1	1	0.12146	0.34653	8346748	7413077	1.1259	1.1174	2.8748	209713392	188672864	1.11152	1.0844	3.0188
	965c-L4-11	13	1	0.12229	0.34832	4899867	4274507	1.1463		2.9219	99350064	88184184	1.12662		3.0547
	965c-L4-12	13	2	0.12217	0.34944	2276935	1971337	1.1550		2.9565	331761696	309499072	1.07193		2.9186
	965c-L4-13	64	1	0.12162	0.34947	5832112	5133451	1.1361		2.9215	281142976	254735120	1.10367		3.0189
5	965c-L4-14	4	1	0.12206	0.35117	7575818	6524614	1.1611	1.1199	2.9828	189016672	175226048	1.07870	1.0548	2.9543
	965c-L4-15	25	1	0.12221	0.35020	9710667	8626910	1.1256		2.8801	351921632	319337568	1.10204		3.0062
	965c-L4-16	50	1	0.12211	0.35045	4366428	3821859	1.1425		2.9278	444345248	406770976	1.09237		2.9843
6	965c-L4-17	18	1	0.12160	0.35051	8432824	7633114	1.1048	1.1240	2.8331	417390656	397188352	1.05086	1.0538	2.8835
	965c-L4-18	40	1	0.12196	0.34990	8534069	7631550	1.1183		2.8542	527427840	504292608	1.04588		2.8564
	965c-L4-19	40	2	0.12174	0.35034	10603957	9385655	1.1298		2.8925	299803200	285850496	1.04881		2.8731
	965c-L4-20	55	1	0.12205	0.34981	6323687	5603372	1.1286		2.8776	400892320	381214592	1.05162		2.8692
								Number of Samples:		20			Number of Samples:		20
								Mean:		2.912			Mean:		2.954
								Standard Deviation		0.044			Standard Deviation		0.071
								Relative Standard Deviation (%)		1.5			Relative Standard Deviation (%)		2.4

5.4.2. Bayesian Analysis

While DMRF is the least complex quantitation approach, calculations that more fully utilize all experimental information, such as measurement uncertainties and repeatabilities, are available. The NIST “Apps for Bayesian Analysis of Chemical quantities Using Shiny)” (ABACUS) Chemical Analysis Package [17] implements methods that use the within-day information. The following OpenBUGS [18] analysis implements the ABACUS internal standard response factor model for the first three days (1, 2, 3) and the last three (4, 5, 6) using 0.002 g as the standard uncertainty in the SRM 917d stocks and 0.00003 g as the standard uncertainty for all mass measurements. The datasets for the two sets of three days are run separately because the stock solutions used differ.

5.4.2.1. OpenBUGS Code

```
# Output
# wd        NS sample mass fractions (run Day123 and Day456 datasets separately)
#
# Inputs
# MT        number of number of sets × samples/set
# MTT       number of sets × samples/set × replicates/sample
# NS        number sets
# NT        number of sets × working standards
# NTT       number of sets × calibrants/set × replicates/calibrant
# NWS       Number working standards
# wsol      NT index of working standard within set
# expc0     NT index of set by calibrant
# expc1     NTT index of set by calibrant by replicate
# expc2     NTT index of calibrant measurement by replicate
# wadm      NWS mass fraction of working standards
# uwad      NWS standard uncertainties for working standard mass fractions
# mids      NT internal standard masses added to calibrants
# umids     NS standard uncertainties of internal standard masses
# mads      NT working standard masses added to calibrants
# umads     NS standard uncertainties for working standard masses
# rac       NTT peak area ratios for calibrants
# expr0     MT index of set by sample
# expr1     MTT index of set by sample by replicate
# expr2     MTT index of sample measurement by replicate
# md        MT serum mass in sample
# ras       MTT peak area ratios for samples
#
Model{
wadmean<-mean(wad[])
for(i in 1:NWS){wadprec[i]<-1/(uwad[i]*uwad[i])
                wad[i]~dnorm(wadm[i],wadprec[i])}
for(i in 1:NS){midsprec[i]<-1/(umids[i]*umids[i])
               madsprec[i]<-1/(umads[i]*umads[i])}
for(i in 1:NT){mids[i]~dnorm(midsm[i],midsprec[expc0[i]])
               mads[i]~dnorm(madsm[i],madsprec[expc0[i]])
               ratio[i]<-mads[i]/mids[i]
               wac[i]<-wad[wsol[i]]* ratio[i]}
#
# Calibration equation
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)
```

```
               fitprec[i]<-xins[i]/sqrt(chsqns[i])}
for(i in 1:NTT){mean[i]<-b[expc1[i]]*wac[expc2[i]]
                rac[i]~dnorm(mean[i],fitprec[expc1[i]])
                predm[i]~dnorm(mean[i],fitprec[expc1[i]])}
#
# Mass fractions
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)
               sigras[i]<-xinsi[i]/sqrt(chsqnsi[i])
               wd[i]~dnorm(0,1.0E-5)}
for(i in 1:MT){rasmean[i]<-b.cut[expr0[i]]*wd[expr0[i]]*md[i]/midsi[i]
               rasmeanp[i]~dnorm(rasmean[i],fitprec[expr0[i]])}
for(i in 1:MTT){ras[i]~dnorm(rasmeanp[expr2[i]],sigras[expr1[i]])}
} End Model
```

5.4.2.2. OpenBUGS Data for Level 1

```
# SRM 965c Level 1 Days 1, 2, 3
list(NWS=2,NS=3,NT=9,NTT=27,
umids=c(0.00003,0.00003,0.00003),
umads=c(0.00003,0.00003,0.00003),
wadm=c(1.210,1.113),uwad=c(0.002,0.002),
wsol=c(1,2,1,2,1,2,1,2,1),
expc0=c(1,1,1,2,2,2,3,3,3),
expc1=c(1,1,1,2,2,2,3,3,3,1,1,1,2,2,2,3,3,3,1,1,1,2,2,2,3,3,3),
expc2=c(1,2,3,4,5,6,7,8,9,1,2,3,4,5,6,7,8,9,1,2,3,4,5,6,7,8,9),
midsm=c(0.20020,0.20116,0.20061,0.19847,0.19818,0.19822,0.20055,0.20111,0.20024),
madsm=c(0.24933,0.20032,0.14971,0.24978,0.20056,0.15078,0.25005,0.20164,0.14888),
rac=c(1.6752,1.2970,0.9939,1.6209,1.3514,0.9785,1.6524,1.2962,0.9856,
      1.6614,1.2906,0.9950,1.6282,1.3564,0.9772,1.6683,1.3025,0.9862,
      1.6593,1.2931,0.9872,1.6196,1.3498,0.9670,1.6544,1.2910,0.9884),
MT=10,MTT=10,
expr0=c(1,1,1,1,2,2,2,3,3,3),
expr1=c(1,1,1,1,2,2,2,3,3,3),
expr2=c(1,2,3,4,5,6,7,8,9,10),
midsi=c(0.08915,0.08896,0.08881,0.08935,0.08946,0.08925,0.08907,0.08986,0.08988,
        0.08997),
md=c(0.24787,0.24602,0.24802,0.24608,0.24461,0.24833,0.24241,0.24503,0.23791,0.24506),
ras=c(1.0345,1.0223,1.0321,1.0221,1.0147,1.0004,1.0101,1.0079,0.9664,1.0339)) End list

# SRM 965c Level 1 Days 4, 5, 6
list(NWS=2,NS=3,NT=9,NTT=27,
umids=c(0.00003,0.00003,0.00003),
umads=c(0.00003,0.00003,0.00003),
wadm=c(1.064,1.341),uwad=c(0.002,0.002),
wsol=c(2,1,2,1,2,1,2,1,2),
expc0=c(1,1,1,2,2,2,3,3,3),
expc1=c(1,1,1,2,2,2,3,3,3,1,1,1,2,2,2,3,3,3,1,1,1,2,2,2,3,3,3),
expc2=c(1,2,3,4,5,6,7,8,9,1,2,3,4,5,6,7,8,9,1,2,3,4,5,6,7,8,9),
midsm=c(0.19924,0.19932,0.20024,0.19987,0.20074,0.20038,0.19964,0.20050,0.20033),
madsm=c(0.25030,0.20072,0.14922,0.24957,0.20113,0.15075,0.20962,0.20063,0.13935),
rac=c(1.8872,1.1852,1.1259,1.4827,1.4998,0.9048,1.5817,1.2075,1.0498,
      1.8818,1.1769,1.1253,1.4802,1.5101,0.8992,1.5797,1.2054,1.0463,
      1.9056,1.1879,1.1234,1.4945,1.5034,0.8923,1.5745,1.2010,1.0382),
MT=9,MTT=9,
expr0=c(1,1,1,2,2,2,3,3,3),
expr1=c(1,1,1,1,2,2,2,3,3,3),
expr2=c(1,2,3,4,5,6,7,8,9,10),
midsi=c(0.09009,0.09032,0.08997,0.08999,0.09002,0.09011,0.08892,0.08888,0.08892),
md=c(0.24535,0.24811,0.2416,0.24592,0.24387,0.24557,0.23863,0.23877,0.24719),
ras=c(0.9866,0.9693,0.9882,1.013,1.0009,1.0067,0.9776,0.9978,1.0113)) End list
```

5.4.2.3. OpenBUGS Data for Level 2

```
# SRM 965c Level 2 Days 1, 2, 3
list(NWS=2,NS=3,NT=9,NTT=27,
umids=c(0.00003,0.00003,0.00003),
umads=c(0.00003,0.00003,0.00003),
wadm=c(1.210, 1.113),uwad=c(0.002, 0.002),
wsol=c(1,2,1,2,1,2,1,2,1),
expc0=c(1,1,1,2,2,2,3,3,3),
expc1=c(1,1,1,2,2,2,3,3,3,1,1,1,2,2,2,3,3,3,1,1,1,2,2,2,3,3,3),
expc2=c(1,2,3,4,5,6,7,8,9,1,2,3,4,5,6,7,8,9,1,2,3,4,5,6,7,8,9),
midsm=c(0.20020,0.20116,0.20061,0.19847,0.19818,0.19822,0.20055,0.20111,0.20024),
madsm=c(0.24933,0.20032,0.14971,0.24978,0.20056,0.15078,0.25005,0.20164,0.14888),
rac=c(1.6752,1.2979,0.9939,1.6209,1.3514,0.9785,1.6524,1.2962,0.9856,
      1.6614,1.2906,0.9950,1.6282,1.3564,0.9772,1.6683,1.3025,0.9862,
      1.6593,1.2931,0.9872,1.6196,1.3498,0.9670,1.6544,1.2910,0.9884),
MT=10,MTT=10,
expr0=c(1,1,1,2,2,2,2,3,3,3),
expr1=c(1,1,1,2,2,2,2,3,3,3),
expr2=c(1,2,3,4,5,6,7,8,9,10),
midsi=c(0.19908,0.19853,0.19932,0.19885,0.19878,0.19922,0.19896,0.19993,0.20008,
        0.20005),
md=c(0.25106,0.24863,0.24957,0.24744,0.24812,0.25212,0.24892,0.24428,0.24632,0.243),
ras=c(1.3402,1.321,1.3079,1.315,1.3149,1.3414,1.3086,1.3412,1.3,1.2712)) End list

# SRM 965c Level 2 Days 4, 5, 6
list(NWS=2,NS=3,NT=9, NTT=27,
umids=c(0.00003,0.00003,0.00003),
umads=c(0.00003,0.00003,0.00003),
wadm=c(1.064,1.341),uwad=c(0.002,0.002),
wsol=c(2,1,2,1,2,1,2,1,2),
expc0=c(1,1,1,2,2,2,3,3,3),
expc1=c(1,1,1,2,2,2,3,3,3,1,1,1,2,2,2,3,3,3,1,1,1,2,2,2,3,3,3),
expc2=c(1,2,3,4,5,6,7,8,9,1,2,3,4,5,6,7,8,9,1,2,3,4,5,6,7,8,9),
midsm=c(0.19924,0.19932,0.20024,0.19987,0.20074,0.20038,0.19964,0.20050,0.20033),
madsm=c(0.25030,0.20072,0.14922,0.24957,0.20113,0.15075,0.20962,0.20063,0.13935),
rac=c(1.8872,1.1852,1.1259,1.4827,1.4998,0.9048,1.5817,1.2075,1.0498,
      1.8818,1.1769,1.1253,1.4802,1.5101,0.8992,1.5797,1.2054,1.0463,
      1.9056,1.1879,1.1234,1.4945,1.5034,0.8923,1.5745,1.2010,1.0382),
MT=10,MTT=10,
expr0=c(1,1,1,2,2,2,2,3,3,3),
expr1=c(1,1,1,2,2,2,2,3,3,3),
expr2=c(1,2,3,4,5,6,7,8,9,10),
midsi=c(0.20106,0.2011,0.20073,0.19894,0.19962,0.19915,0.198899,0.20034,0.20068,
        0.19977),
md=c(0.24676,0.24535,0.24573,0.24591,0.24579,0.24334,0.244191,0.24885,0.24484,
     0.24593),
ras=c(1.2887,1.2816,1.2956,1.381,1.2458,1.2856,1.2575,1.297,1.2809,1.2666)) end list
```

5.4.2.4. OpenBUGS Data for Level 3

```
# SRM 965c Level 3 Days 1, 2, 3
list(NWS=2,NS=3,NT=9,NTT=27,
umids=c(0.00003,0.00003,0.00003),
umads=c(0.00003,0.00003,0.00003),
wadm=c(1.210,1.113),uwad=c(0.002,0.002),
wsol=c(1,2,1,2,1,2,1,2,1),
expc0=c(1,1,1,2,2,2,3,3,3),
expc1=c(1,1,1,2,2,2,3,3,3,1,1,1,2,2,2,3,3,3,1,1,1,2,2,2,3,3,3),
expc2=c(1,2,3,4,5,6,7,8,9,1,2,3,4,5,6,7,8,9,1,2,3,4,5,6,7,8,9),
midsm=c(0.20020,0.20116,0.20061,0.19847,0.19818,0.19822,0.20055,0.20111,0.20024),
```

```
madsm=c(0.24933,0.20032,0.14971,0.24978,0.20056,0.15078,0.25005,0.20164,0.14888),
rac=c(1.6752,1.2970,0.9939,1.6209,1.3514,0.9785,1.6524,1.2962,0.9856,
      1.6614,1.2906,0.9950,1.6282,1.3564,0.9772,1.6683,1.3025,0.9862,
      1.6593,1.2931,0.9872,1.6196,1.3498,0.9670,1.6544,1.2901,0.9884),
MT=10,MTT=10,
expr0=c(1,1,1,2,2,2,2,3,3,3),
expr1=c(1,1,1,2,2,2,3,3,3,3),
expr2=c(1,2,3,4,5,6,7,8,9,10),
midsi=c(0.29725,0.29909,0.29899,0.29458,0.30214,0.2958,0.30003,0.3002,0.29942,
        0.29949),
md=c(0.24461,0.25040,0.25100,0.24817,0.24865,0.24922,0.24543,0.24596,0.23889,
     0.23924),
ras=c(1.1311,1.1477,1.1464,1.1391,1.1066,1.1233,1.0993,1.1488,1.0535,1.0622)) End list

# SRM 965c Level 3 Days 4, 5, 6
list(NWS=2,NS=3,NT=9,NTT=27,
umids=c(0.00003,0.00003,0.00003),
umads=c(0.00003,0.00003,0.00003),
wadm=c(1.064,1.341),uwad=c(0.002,0.002),
wsol=c(2,1,2,1,2,1,2,1,2),
expc0=c(1,1,1,2,2,2,3,3,3),
expc1=c(1,1,1,2,2,2,3,3,3,1,1,1,2,2,2,3,3,3,1,1,1,2,2,2,3,3,3),
expc2=c(1,2,3,4,5,6,7,8,9,1,2,3,4,5,6,7,8,9,1,2,3,4,5,6,7,8,9),
midsm=c(0.19924,0.19932,0.20024,0.19987,0.20074,0.20038,0.19964,0.2005,0.20033),
madsm=c(0.2503,0.20072,0.14922,0.24957,0.20113,0.15075,0.20962,0.20063,0.13935),
rac=c(1.8872,1.1852,1.1259,1.4827,1.4998,0.9048,1.5817,1.2075,1.0498,
      1.8818,1.1769,1.1253,1.4802,1.5101,0.8992,1.5797,1.2054,1.0463,
      1.9056,1.1879,1.1234,1.4945,1.5034,0.8923,1.5745,1.201,1.0382),
MT=9,MTT=9,
expr0=c(1,1,1,2,2,2,3,3,3),
expr1=c(1,1,1,2,2,2,3,3,3),
expr2=c(1,2,3,4,5,6,7,8,9),
midsi=c(0.29991,0.2997,0.30025,0.2999,0.29891,0.2992,0.2992,0.29999,0.2995),
md=c(0.24486,0.24608,0.24644,0.24543,0.24508,0.2404,0.24053,0.24725,0.248),
ras=c(1.1074,1.1145,1.1088,1.0764,1.1069,1.0963,1.084,1.1172,1.1295)) End list
```

5.4.2.5. OpenBUGS Data for Level 4

```
# SRM 965c Level 4 Days 1, 2, 3
list(NWS=2,NS=3,NT=9,NTT=27,
umids=c(0.00003,0.00003,0.00003),
umads=c(0.00003,0.00003,0.00003),
wadm=c(1.210,1.113),uwad=c(0.002,0.002),
wsol=c(1,2,1,2,1,2,1,2,1),
expc0=c(1,1,1,2,2,2,3,3,3),
expc1=c(1,1,1,2,2,2,3,3,3,1,1,1,2,2,2,3,3,3,1,1,1,2,2,2,3,3,3),
expc2=c(1,2,3,4,5,6,7,8,9,1,2,3,4,5,6,7,8,9,1,2,3,4,5,6,7,8,9),
midsm=c(0.2002,0.20116,0.20061,0.19847,0.19818,0.19822,0.20055,0.20111,0.20024),
madsm=c(0.24933,0.20032,0.14971,0.24978,0.20056,0.15078,0.25005,0.20164,0.14888),
rac=c(1.6752,1.297,0.9939,1.6209,1.3514,0.9785,1.6524,1.2962,0.9856,
      1.6614,1.2906,0.995,1.6282,1.3564,0.9772,1.6683,1.3025,0.9862,
      1.6593,1.2931,0.9872,1.6196,1.3498,0.967,1.6544,1.291,0.9884),
MT=9,MTT=9,
expr0=c(1,1,1,2,2,2,3,3,3),
expr1=c(1,1,1,2,2,2,3,3,3),
expr2=c(1,2,3,4,5,6,7,8,9),
midsi=c(0.34897,0.34906,0.35002,0.35164,0.34881,0.3478,0.34979,0.3496,0.34973),
md=c(0.1195,0.11478,0.12145,0.12282,0.12312,0.12226,0.12169,0.12148,0.12169),
ras=c(1.1363,1.0888,1.1604,1.1579,1.1556,1.1621,1.1092,1.1482,1.1498)) End list

# SRM 965c Level 4 Days 4, 5, 6
list(NWS=2,NS=3,NT=9,NTT=27,
```

```
umids=c(0.00003,0.00003,0.00003),
umads=c(0.00003,0.00003,0.00003),
wadm=c(1.064,1.341),uwad=c(0.002,0.002),
wsol=c(2,1,2,1,2,1,2,1,2),
expc0=c(1,1,1,2,2,2,3,3,3),
expc1=c(1,1,1,2,2,2,3,3,3,1,1,1,2,2,2,3,3,3,1,1,1,2,2,2,3,3,3),
expc2=c(1,2,3,4,5,6,7,8,9,1,2,3,4,5,6,7,8,9,1,2,3,4,5,6,7,8,9),
midsm=c(0.19924,0.19932,0.20024,0.19987,0.20074,0.20038,0.19964,0.2005,0.20033),
madsm=c(0.2503,0.20072,0.14922,0.24957,0.20113,0.15075,0.20962,0.20063,0.13935),
rac=c(1.8872,1.1852,1.1259,1.4827,1.4998,0.9048,1.5817,1.2075,1.0498,
      1.8818,1.1769,1.1253,1.4802,1.5101,0.8992,1.5797,1.2054,1.0463,
      1.9056,1.1879,1.1234,1.4945,1.5034,0.8923,1.5745,1.201,1.0382),
MT=11,MTT=11,
expr0=c(1,1,1,1,2,2,2,3,3,3,3),
expr1=c(1,1,1,1,2,2,2,3,3,3,3),
expr2=c(1,2,3,4,5,6,7,8,9,10,11),
midsi=c(0.34832,0.34944,0.34653,0.34947,0.35045,0.35117,0.3502,0.3499,0.35034,0.34981,
        0.35051),
md=c(0.12229,0.12217,0.12146,0.12162,0.12211,0.12206,0.12221,0.12196,0.12174,
     0.12205,0.1216),
ras=c(1.1463,1.1550,1.1259,1.1361,1.1425,1.1611,1.1256,1.1183,1.1298,
      1.1286,1.1048)) End list
```

5.4.2.6. Mass Fraction Estimates

Table 15 lists the glucose mass fraction estimates for the six days for each of the four SRM 965c Levels calculated using the OpenBUGS Bayesian analysis. The consensus value and its standard uncertainty are calculated using the NICOB system's [12] Hierarchical Bayes (Gaussian) tool. The NICOB analyses are displayed in Fig. 11.

Table 15. OpenBUGS Estimates of SRM 965c Glucose Mass Fractions, mg/g.

	Level 1		Level 2		Level 3		Level 4	
Day	w_{965c}	$u(w_{965c})$	w_{965c}	$u(w_{965c})$	w_{965c}	$u(w_{965c})$	w_{965c}	$u(w_{965c})$
1	0.330	0.017	0.939	0.060	1.220	0.090	2.96	0.22
2	0.322	0.026	0.926	0.033	1.178	0.052	2.89	0.20
3	0.332	0.029	0.954	0.064	1.211	0.077	2.92	0.23
4	0.322	0.023	0.942	0.044	1.206	0.070	2.91	0.10
5	0.330	0.012	0.934	0.043	1.202	0.077	2.92	0.18
6	0.327	0.021	0.926	0.044	1.205	0.063	2.86	0.07
Consensus	0.328		0.934		1.204		2.89	
u(Consensus)	0.008		0.017		0.027		0.05	
U_{95}(Consensus) [a]	0.016		0.034		0.054		0.10	
u_{rel}(Consensus) [b]	2.4 %		1.8 %		2.2 %		1.7 %	

a 95 % level of confidence expanded uncertainty, calculated as one-half of the 95 % credible interval

b Relative standard uncertainty, calculated as $100*u(w_{965c})/w_{965c}$

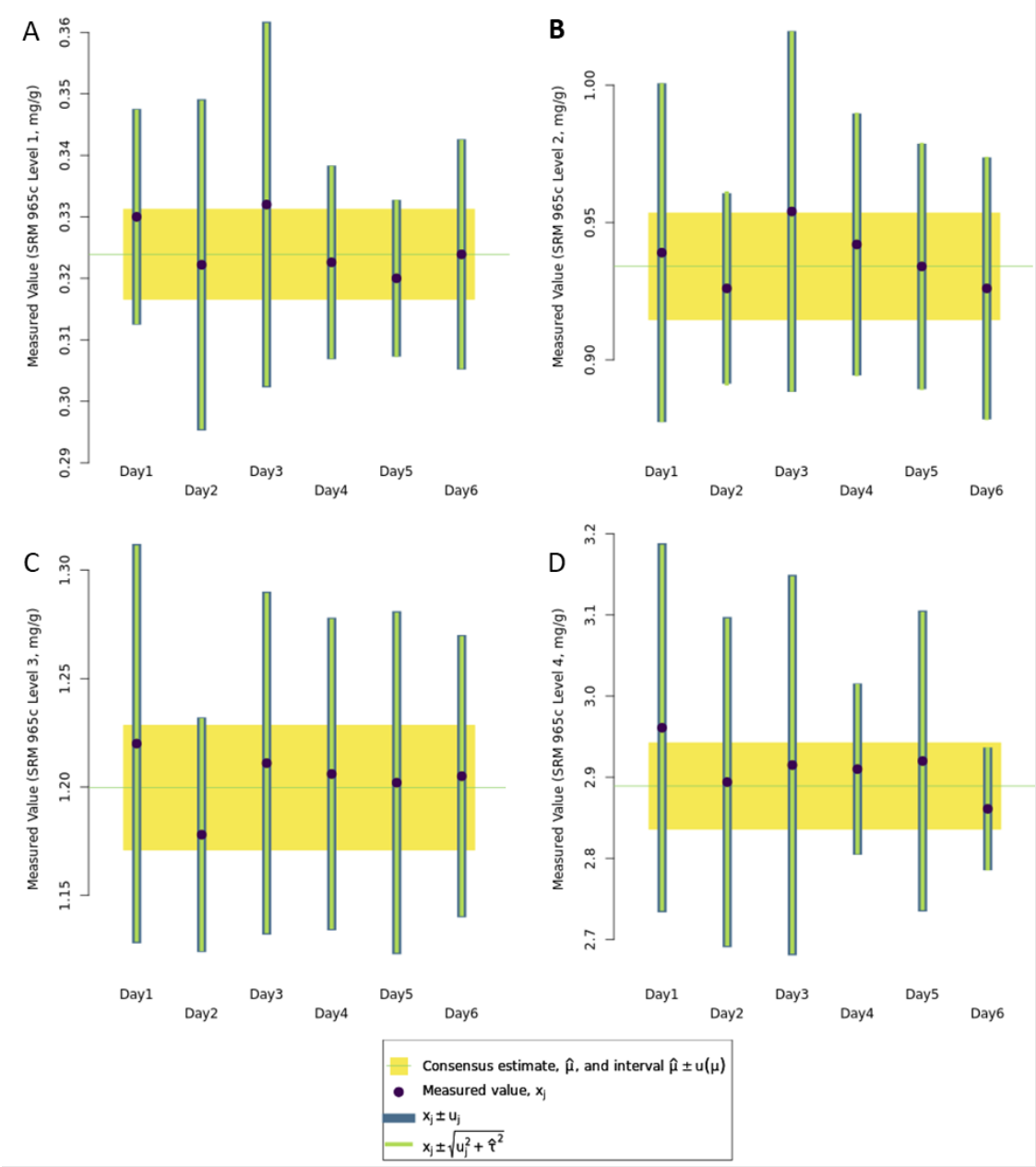

Fig. 11. NIST Consensus Glucose Mass Fraction Estimates for SRM 965c Levels 1 Through 4.

Each dot denotes an estimate of the mean glucose mass fraction in three SRM 965c ampoules. The bars denote the span of the estimated standard uncertainties of the mean value. Panel A displays the Level 1 results, Panel B the Level 2 results, Panel C the Level 3 results, and Panel D the Level 4 results.

6. Quality Assurance

As shown in Fig. 12, the DMRF results for SRM 965b listed in Table 10 for both the RMP and the Oribitrap method are in excellent agreement with the certified values and with each other.

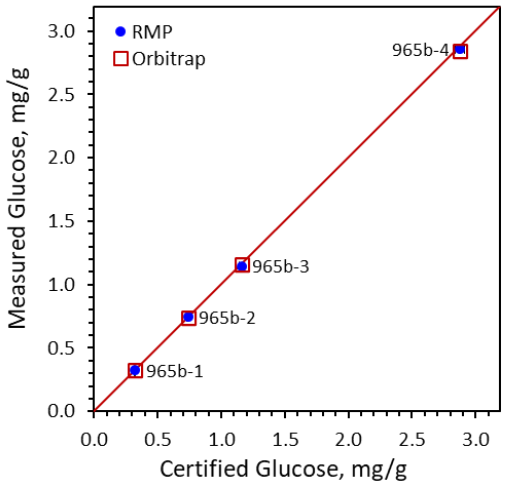

Fig. 12. Measured Results for SRM 965b as a Function of Certified Values.

Each symbol represents a daily mean response factor (DMRF) estimate of the glucose concentration result for one level of SRM 965b as a function of its certified concentration. Solid circles denote results from the certification method; open squares denote results from the confirmation method. The diagonal line represents equality between the measured and certified values.

The agreement between the measured and certified values confirms that the RMP's calibrant and sample preparation stages are not significant sources of measurement bias. The agreement between the results provided by the RMP and exploratory Orbitrap method confirms that the separation and detection stages of the RMP are also not significant sources of measurement bias.

While the quality of the Orbitrap results suggests that the method holds promise, only results provided by the RMP are appropriate for use in certifying serum glucose concentrations in the SRM 965c materials.

7. Homogeneity

The DMRF glucose concentration results for the four SRM 965c Levels listed in Table 11, Table 12, Table 13, and Table 14 are displayed in Fig. 13 as functions of storage Box (alias for the within-level ampoule production sequence) and analysis Day (alias for between-day analysis sequence).

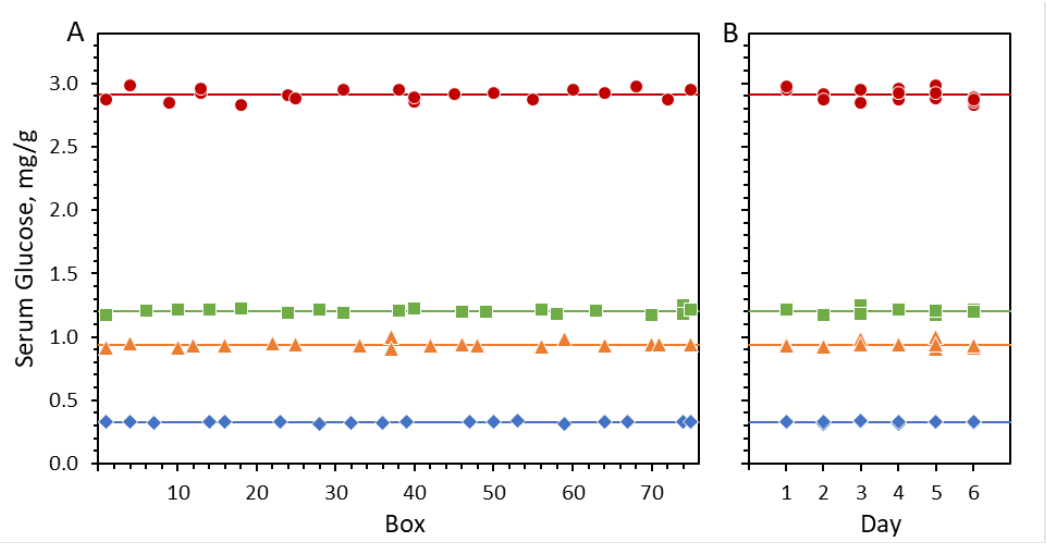

Fig. 13. Homogeneity Assessment for Production and Analysis Session Orders.

Each symbol represents one replicate measurement of an ampoule drawn from one of the 76 storage boxes of one of the four SRM 965c Levels. Diamonds denote results for Level 1; triangles for Level 2; squares for Level 3, and circles for Level 4. Horizontal lines denote the mean values for each set of measurements. Panel A displays the results as functions of within-level ampoule production storage box number. Panel B displays the results as functions of the six measurement sessions required to process the 78 ampoules.

There are no apparent trends with Box or Day. The between-ampoule and within-ampoule variabilities are of similar magnitude.

The Cochran Q [19] test of homogeneity with respect to boxes indicates that there is no evidence of between-Box heterogeneity for any of the four materials.

8. CDC Monosaccharide Determinations

CDC's Clinical Reference Laboratory (CDC CRL), Division of Laboratory Sciences, National Center for Environmental Health evaluated the concentrations of glucose, fructose, and mannose in the four SRM 965c materials.

SRM 965c was designed to provide a range of glucose values across four levels of material. Since fructose and mannose were not considered at the time of SRM 965c preparation, the levels for these two monosaccharides are the result of the pooling and processing that was used to target specific glucose concentration ranges.

8.1. Analyses

Thirteen ampoules from unspecified batch positions for each of SRM 965c Level 1, Level 2, Level 3, and Level 4 were shipped on dry ice overnight from the NIST Office of Reference Materials to the CDC.

8.1.1. Glucose

The four SRM 965c materials were analyzed for glucose in three independent runs using the CDC's established Isotope Dilution-Gas Chromatographic-Mass Spectroscopy (ID-GC-MS) reference measurement procedure (RMP) [7].

A set of samples containing four levels of NIST SRM 965c was prepared. Samples were accurately weighed and spiked gravimetrically with labeled internal standard (D-glucose-$^{13}C_6$, Sigma-Aldrich). Analysis was performed using an 8890/5977B Agilent GCMS system in three independent runs for all levels of NIST 965c. Each independent run contained four independent preparations from one ampoule (within vial replicates) of each of the four levels of SRM 965c.

The glucose measurements were calibrated with NIST SRM 917c D-Glucose (Dextrose) [9] using a 6-point calibration curve spiked with D-glucose-$^{13}C_6$. Each calibrant was analyzed in duplicate.

Three levels of SRM 965b were analyzed as trueness controls with each independent run (SRM 965b Levels 1, 4, and one of Levels 2 and 3 interchangeably). All results for these materials were within the certified ranges.

8.1.2. Fructose and Mannose

The four SRM 965c materials were analyzed for fructose and mannose using an Isotope Dilution-Liquid Chromatography-Tandem Mass spectroscopy (ID-LC-MS/MS) method developed by the CDC [20,21,22,23].

A set of samples containing four levels of NIST 965c materials was prepared. Samples and calibrators were spiked with labeled internal standards (D-fructose-$^{13}C_6$ and D-mannose-$^{13}C_6$, Sigma-Aldrich). Analysis was performed using a Shimadzu Nexera X2 LC-system coupled with a Sciex Triple Quad 6500+ MS system in three independent runs for all levels of NIST 965c. Each

independent run contained four independent preparations from one ampoule of each of the four levels of SRM 965c.

The fructose and mannose measurements were calibrated with primary reference standards (fructose: USP #1286504; mannose: USP #1375182) using a 6-point calibration curve. Each calibrant was analyzed in duplicate. In-house prepared, serum-based Quality Control materials were used since no reference materials for fructose or mannose in serum were available.

8.2. CDC Supplied Values

The CDC prepared samples gravimetrically for glucose and volumetrically for fructose and mannose. Glucose mass fractions were measured as mg/g and converted to mg/dL units using NIST-measured density values. Fructose and mannose concentrations were measured as mass concentrations with units of mg/dL. For each level of SRM 965c, CDC provided the number of measurements (n), sample mean ($x_{analyte}$), and standard deviation ($s_{analyte}$) for glucose, fructose, and mannose. Assuming that the measurements were gaussian distributed, the expanded uncertainty can be based on the student t distribution with n-1 degrees of freedom. In all cases n = 12, so the critical value for 95% uncertainty interval is 2.20. These values are reported Table 16.

Table 16. CDC Mass Concentration Results for Glucose, Fructose, and Mannose in SRM 965c, mg/dL.

		Glucose			Fructose			Mannose		
Level	n	x_{965c}	s_{965c}	$U_{95}(x_{965c})$[a]	x_{965c}	s_{965c}	$U_{95}(x_{965c})$[a]	x_{965c}	s_{965c}	$U_{95}(x_{965c})$[a]
1	12	33.61	0.35	0.77	0.36	0.03	0.07	1.00	0.04	0.09
2	12	96.03	0.72	1.58	0.31	0.02	0.04	1.16	0.05	0.11
3	12	124.63	0.94	2.07	0.36	0.03	0.07	1.37	0.04	0.09
4	12	300.16	2.05	4.51	0.38	0.04	0.09	1.40	0.04	0.09

a 95 % level of confidence expanded uncertainty, calculated as $2.2 \times s_{965c}$

9. Consensus Glucose Mass Fractions

The NIST (single preparations, plus on duplicate preparation from 17 ampoules) and CDC (four preparations from each of 3 ampoules) results for glucose in the SRM 965c materials can be combined using the DerSimonian-Laird uncertainty-weighed mean as implemented in the NICOB [12]. The resulting distributions for glucose in the SRM 965c materials are displayed in Table 16. The mass fraction results are listed in Table 17.

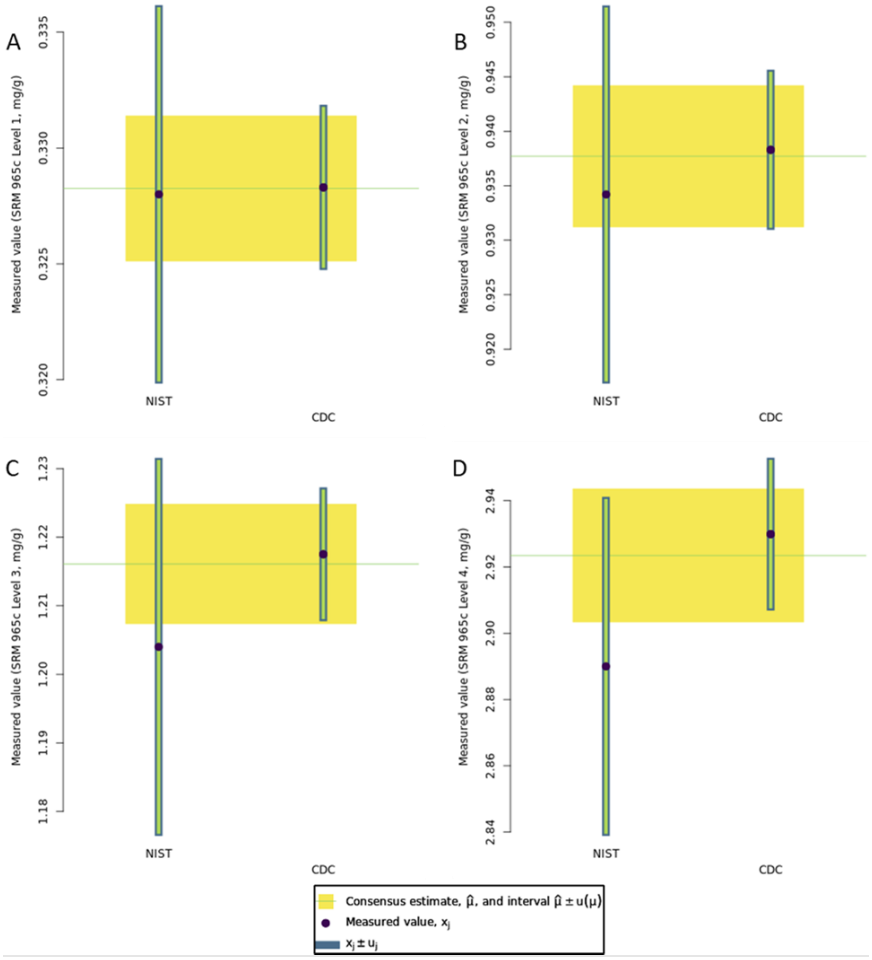

Fig. 14. {NIST,CDC} Consensus Glucose Mass Fraction Estimates for SRM 965c Levels 1 Through 4.

Each dot denotes a consensus estimate of the NIST and CDC results for glucose mass fraction in the SRM 965c materials. The bars denote the span of the estimated standard uncertainties of the consensus values. The insets to each panel provide the estimated consensus parameters. Panel A displays the Level 1 results, Panel B the Level 2 results, Panel C the Level 3 results, and Panel D the Level 4 results.

Table 17. Consensus Mass Fraction Glucose Results for SRM 965c Levels 1 Through 4.

	Level 1			Level 2			Level 3			Level 4		
	w_{965c}	$u(w_{965c})$	U_{95}[a]	w_{965c}	$u(w_{965c})$	U_{95}[a]	w_{965c}	$u(w_{965c})$	U_{95}[a]	w_{965c}	$u(w_{965c})$	U_{95}[a]
NIST	0.328	0.008	0.016	0.934	0.017	0.034	1.204	0.027	0.054	2.89	0.05	0.10
CDC	0.3283	0.0034	0.0068	0.938	0.007	0.014	1.2175	0.0092	0.018	2.9299	0.022	0.044
Combined	0.328	0.003	0.006	0.937	0.006	0.012	1.214	0.009	0.018	2.92	0.02	0.04

a 95 % level of confidence expanded uncertainty

10. Unit Transformations

NIST's measurements were made using gravimetric preparation and reported as mass fractions (w_{analyte}) in units of mg/g. The clinical communities are more familiar with values expressed as mass concentrations (x_{analyte}) in units of g/dL and amount-of-substance concentrations (c_{analyte}) in units of mmol/L. Using the measured serum densities (ρ_{23}) described in Section 3 and the molar mass (molecular weight) of $C_6H_{12}O_6$ monosaccharides (M_{analyte}) described in Section 4, the following equations describe the units transformations.

Note: Glucose, fructose, and mannose have the same molecular formula and therefore the same M_{analyte} of 180.156 g/mol and large-sample standard uncertainty $u(M_{\text{analyte}})$ of 0.004 g/mol.

10.1. Mass Concentration to Mass Fraction and Amount-of-Substance Concentration

The CDC supplied results as mass concentrations (x_{analyte}) in units of mg/dL. The following converts these results to mass fractions (w_{analyte}) with units of mg/g:

$$w_{\text{analyte}}\left(\frac{\text{mg}}{\text{g}}\right) = x_{\text{analyte}}\left(\frac{\text{g}}{\text{dL}}\right)\left(\frac{\text{dL}}{100\ \text{mL}}\right)/\rho_{23}\left(\frac{\text{g}}{\text{mL}}\right) \tag{13}$$

Given the lack of detailed experimental information, the CDC's s_{analyte} serve as estimates of large-sample standard uncertainties ($u(x_{\text{analyte}})$). Using one-half of the half-width of the 95 % credible interval of the DerSimonian-Laird estimated serum densities as "large sample" standard uncertainties ($u(\rho_{23})$), the mass fraction standard uncertainties ($u(w_{\text{analyte}})$) can be estimated using the first order (Gauss's Law) uncertainty equation [11]:

$$u\left(w_{\text{analyte}}\right) = w_{\text{analyte}}\sqrt{\left(\frac{u\left(x_{\text{analyte}}\right)}{x_{\text{analyte}}}\right)^2 + \left(\frac{u(\rho_{23})}{\rho_{23}}\right)^2}. \tag{14}$$

Similarly, the following converts mass concentrations in units of mg/dL to amount-of-substance concentrations (c_{analyte}) in units of mmol/L:

$$c_{\text{analyte}}\left(\frac{\text{mmol}}{\text{L}}\right) = x_{\text{analyte}}\left(\frac{\text{mg}}{\text{dL}}\right)\left(\frac{\text{g}}{1000\ \text{mg}}\frac{1000\ \text{mmol}}{\text{mol}}\frac{10\ \text{dL}}{\text{L}}\right)/M_{\text{analyte}}\left(\frac{\text{g}}{\text{mol}}\right) \tag{15}$$

$$u\left(c_{\text{analyte}}\right) = c_{\text{analyte}}\sqrt{\left(\frac{u\left(w_{\text{analyte}}\right)}{w_{\text{analyte}}}\right)^2 + \left(\frac{u\left(M_{\text{analyte}}\right)}{M_{\text{analyte}}}\right)^2}. \tag{16}$$

10.2. Mass Fraction to Mass Concentration and Amount-of-Substance Concentration

The following converts NIST's mass fractions (w_{analyte}) in units of mg/g to mass concentrations (x_{analyte}) in units of mg/dL:

$$x_{\text{analyte}}\left(\frac{\text{mg}}{\text{dL}}\right) = w_{\text{analyte}}\left(\frac{\text{mg}}{\text{g}}\right)\rho_{23}\left(\frac{\text{g}}{\text{mL}}\right)\left(\frac{100\ \text{mL}}{\text{dL}}\right) \tag{17}$$

$$u\left(x_{\text{analyte}}\right) = x_{\text{analyte}}\sqrt{\left(\frac{u\left(w_{\text{analyte}}\right)}{w_{\text{analyte}}}\right)^2 + \left(\frac{u\left(\rho_{23}\right)}{\rho_{23}}\right)^2}. \quad (18)$$

Similarly, the following converts mass fractions in units of mg/g to amount-of-substance concentrations (c_{analyte}) in units of mmol/L:

$$c_{\text{analyte}}\left(\frac{\text{mmol}}{\text{L}}\right) = w_{\text{analyte}}\left(\frac{\text{mg}}{\text{g}}\right)\rho_{23}\left(\frac{\text{g}}{\text{mL}}\right)\left(\frac{\text{g}}{1000\ \text{mg}}\frac{1000\ \text{mmol}}{\text{mol}}\frac{1000\ \text{mL}}{\text{L}}\right)/M_{\text{analyte}}\left(\frac{\text{g}}{\text{mol}}\right) \quad (19)$$

$$u\left(c_{\text{analyte}}\right) = c_{\text{analyte}}\sqrt{\left(\frac{u\left(w_{\text{analyte}}\right)}{w_{\text{analyte}}}\right)^2 + \left(\frac{u\left(\rho_{23}\right)}{\rho_{23}}\right)^2 + \left(\frac{u\left(M_{\text{analyte}}\right)}{M_{\text{analyte}}}\right)^2}. \quad (20)$$

10.3. Summary Results

Table 18 and a 95 % level of confidence expanded uncertainty

Table 19 summarize the CDC results for fructose and mannose in the four SRM 965c materials as mass fractions, mass concentrations, and amount-of-substance concentrations.

Table 18. CDC Summary Results for Fructose in SRM 965c Levels 1 Through 4.

	mg/g			mg/dL			mmol/L		
Level	w_{965c}	$u(w_{965c})$	U_{95}[a]	x_{965c}	$u(x_{965c})$	U_{95}[a]	c_{965c}	$u(c_{965c})$	U_{95}[a]
1	0.0036	0.0003	0.0007	0.36	0.03	0.07	0.020	0.002	0.004
2	0.0031	0.0002	0.0004	0.31	0.02	0.04	0.017	0.001	0.002
3	0.0036	0.0003	0.0007	0.36	0.03	0.07	0.020	0.002	0.004
4	0.0038	0.0004	0.0009	0.38	0.04	0.09	0.021	0.002	0.005

a 95 % level of confidence expanded uncertainty

Table 19. CDC Summary Results for Mannose in SRM 965c Levels 1 Through 4.

	mg/g			mg/dL			mmol/L		
Level	w_{965c}	$u(w_{965c})$	U_{95}[a]	x_{965c}	$u(x_{965c})$	U_{95}[a]	c_{965c}	$u(c_{965c})$	U_{95}[a]
1	0.0098	0.0004	0.0009	1.00	0.04	0.09	0.056	0.002	0.005
2	0.0113	0.0005	0.0011	1.16	0.05	0.11	0.064	0.003	0.006
3	0.0134	0.0004	0.0009	1.37	0.04	0.09	0.076	0.002	0.005
4	0.0137	0.0004	0.0009	1.40	0.04	0.09	0.078	0.002	0.005

a 95 % level of confidence expanded uncertainty

Table 20 summarizes the NIST, CDC, and consensus values for glucose in the four SRM 965c materials as mass fractions, mass concentrations, and amount-of-substance concentrations.

Table 20. Units Transformations for Serum Glucose in SRM 965c Levels 1 Through 4.

		mg/g			mg/dL			mmol/L		
Level	Source	w_{965c}	$u(w_{965c})$	U_{95}[a]	x_{965c}	$u(x_{965c})$	U_{95}[a]	c_{965c}	$u(c_{965c})$	U_{95}[a]
1	NIST	0.328	0.008	0.016	33.58	0.82	1.64	1.864	0.046	0.091
	CDC	0.3283	0.0034	0.0068	33.61	0.35	0.70	1.866	0.019	0.039
	Combined	0.3280	0.0031	0.0062	33.58	0.32	0.63	1.864	0.018	0.036
2	NIST	0.934	0.017	0.034	95.61	1.74	3.48	5.307	0.097	0.194
	CDC	0.9383	0.0070	0.0140	96.028	0.717	1.434	5.330	0.0398	0.0796
	Combined	0.9374	0.0060	0.0120	95.936	0.614	1.228	5.325	0.0340	0.0680
3	NIST	1.204	0.027	0.054	123.2	2.8	5.5	6.844	0.153	0.306
	CDC	1.2175	0.0092	0.0184	124.63	0.94	1.89	6.918	0.052	0.105
	Combined	1.214	0.009	0.018	124.24	0.92	1.84	6.897	0.051	0.102
4	NIST	2.89	0.05	0.10	296.1	5.1	10.2	16.43	0.28	0.57
	CDC	2.9299	0.0219	0.0438	300.16	2.05	4.1	16.661	0.114	0.228
	Combined	2.92	0.02	0.04	299.14	2.05	4.1	16.605	0.11	0.22

References

[1] Ashenhurst J (2023) Pyranoses and Furanoses: Ring-Chain Tautomerism In Sugars. https://www.masterorganicchemistry.com/2017/07/13/pyranoses-and-furanoses-ring-chain-tautomerism-in-sugars/. Accessed 11/19/2024.

[2] Marks V (1996) Blood glucose: its measurement and clinical importance. Clin Chim Acta 251:3-17. https://doi.org/10.1016/0009-8981(96)06330-9.

[3] National Institute of Standards and Technology (December 18, 1996) Certificate of Analysis, Standard Reference Material 965 Glucose in Frozen Human Serum. https://tsapps.nist.gov/srmext/certificates/archives/965. Dec 18, 1996.pdf.

[4] National Institute of Standards and Technology (29 September 2008) Certificate of Analysis, Standard Reference Material 965a Glucose in Frozen Human Serum. https://tsapps.nist.gov/srmext/certificates/archives/965a.pdf.

[5] National Institute of Standards and Technology (1 August 2019) Certificate of Analysis, Standard Reference Material 965b Glucose in Frozen Serum. https://tsapps.nist.gov/srmext/certificates/965b.pdf.

[6] Zhang T, Zhang C, Zhao H, Zeng J, Zhang J, Zhou W, Yan Y, Wang Y, Wang M, Chem W (2016) Determination of serum glucose by isotope dilution liquid chromatography-tandem mass spectrometry: a candidate reference measurement procedure. Anal Bioanal Chem 408:7403-7411. https://doi.org/10.1007/s00216-016-9817-0.

[7] Dahya K, Kuiper HC, Kingsley SW, Danilenko U, Vesper HW (2025) Development of an isotope dilution gas chromatography mass spectrometry candidate Reference Measurement Procedure for glucose in human serum. Submitted to JMSACL.

[8] Joint Committee for Traceability in Laboratory Medicine (JCTLM) DB Identifier C4RMP1, https://www.jctlmdb.org/#/app/search/search-results?keywords=C4RMP1&code_status=P.

[9] National Institute of Standards and Technology (18 January 2023) Certificate of Analysis, Standard Reference Material 917d D-Glucose (Dextrose). https://tsapps.nist.gov/srmext/certificates/917d.pdf.

[10] Sniegoski LT, Moody JR (1979) Determination of serum and blood densities. Anal Chem 51(9):1577-1578. https://doi.org/10.1021/ac50045a052.

[11] International Organization for Standardization Joint Committee for Guides in Metrology (2008) JCGM 100:2008 (2008) Evaluation of measurement data — Guide to the expression of uncertainty in measurement (ISO GUM 1995 with minor corrections) (ISO, Geneva). https://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf.

[12] NIST Consensus Builder (NICOB). https://consensus.nist.gov. The user's manual is available at https://consensus.nist.gov/app_direct/nicob/NISTConsensusBuilder-UserManual.pdf. See also: Koepke A, Lafarge T, Possolo A, Toman B (2017) Consensus building for interlaboratory studies, key comparisons, and meta-analysis. Metrologia 54:S34. https://doi.org/10.1088/1681-7575/aa6c0e.

[13] Thompson M, Ellison SLR (2011) Dark uncertainty. Accred Qual Assur 16:483-487. https://doi.org/10.1007/s00769-011-0803-0.

[14] Juris Meija (2016-2019) IUPAC Molecular Weight Calculator, ciaaw.org [BETA release v.0.7, May 2019]. Commission on Isotopic Abundances and Atomic Weights (CIAAW), International Union of Pure and Applied Chemistry (IUPAC) Project 2015-037-2-200. https://ciaaw.shinyapps.io/calculator.

[15] Possolo A, van der Veen AMH, Meija J, Hibbert DB (2018) Interpreting and propagating the uncertainty of the standard atomic weights (IUPAC Technical Report). Pure Appl Chem 90:395-424. https://doi.org/10.1515/pac-2016-0402.

[16] White VE, Welch MJ, Sun T, Sniegoski LT, Schaffer R, Hertz HS, Cohen A (1982) The Accurate Determination of Serum Glucose by Isotope Dilution Mass Spectrometry - Two Methods. Biomed Mass Spectrom;9:395–405. https://doi.org/10.1002/bms.1200090907.

[17] NIST ABACUS (Apps for Bayesian Analysis of Chemical quantities Using Shiny) Chemical Analysis Package. https://abacus.nist.gov/.

[18] Lunn D, Spiegelhalter DJ, Thomas A, Best N (2009). "The BUGS Project: Evolution, Critique and Future Directions." Statistics in Medicine, 28(25), 3049–3067. https://doi.org/10.1002/sim.3680.

[19] Cochran WG (1954) The combination of estimates from different experiments. Biometrics;10(1):101–129. https://doi.org/10.2307/3001666.

[20] Tse CY, Zhang L, Dahya K, Pokuah F, Sugahara O, Danilenko U, Vesper HW (2022) ODP179 Development of Derivatization-free ID-LC/MS/MS Method for Simultaneous Measurement of Human Serum Monosaccharides, Endocr J;6(S1):A305. https://doi.org/10.1210/jendso/bvac150.631.

[21] Tse CY, Li Z, Dahya K, Pokuah F, Sugahara O, Danilenko U, Vesper, H (2023) High Throughput Derivatization-free ID-LC/MS/MS Method for Simultaneous Quantification of Monosaccharides in Human Serum. Mass Spectrometry & Advances in the Clinical Lab (MSACL) Annual Meeting 2023. https://www.msacl.org/program/view_abstract_selection.php?id=2051&event=2023.

[22] Tse CY, Li Z, Dahya K, Pokuah F, Sugahara O, Danilenko U, Delatour V, Vesper H (2023) High Throughput ID-LC/MS/MS Method for Simultaneous Quantitation of Monosaccharides in Human Serum. Association for Diagnostics and Laboratory Medicine (ADLM) Annual Meeting 2023. Clin Chem 69(S1) https://doi.org/10.1093/clinchem/hvad097.516.

[23] Tse CY, Li Z, Dahya K, Pokuah F, Sugahara O, Danilenko U, Delatour V, Vesper H (2023) Comparison between ID-LC/MS/MS Serum Monosaccharides Method and Enzymatic Methods on Glucose Measurements. Diabetes Technology Meeting (DTS) Annual Meeting 2023

Appendix A. List of Acronyms

ABACUS	Apps for Bayesian Analysis of Chemical quantities Using Shiny
CDC	The Centers for Disease Control and Prevention
HPLC	high performance liquid chromatography
ID	isotope dilution
IRB	Institutional Review Board
IS	internal standard
JCTLM	Joint Committee for Traceability in Laboratory Medicine
LC	liquid chromatography
LC-MS	liquid chromatography mass spectrometry
MS	mass spectrometry
MS/MS	tandem mass spectrometry
NICOB	NIST Consensus Builder
NIST	National Institute of Standards and Technology
OHRP	United States Department of Health and Human Services' Office for Human Research Protections
RMP	reference measurement procedure
RF	response factor
SD	standard deviation
SI	International System of Units (Système international d'unités)
SRM®	Standard Reference Material®
TPOC	Technical Point of Contact