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Certification of Standard Reference Material® 3666

Albumin and Creatinine in Frozen Human Urine

Ashley Beasley-Green
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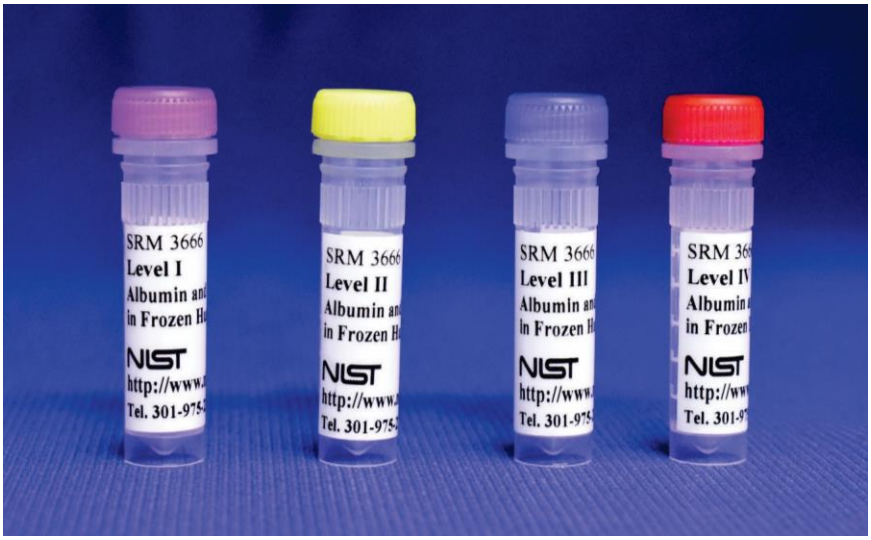

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Ashley Beasley-Green
Biomolecular Measurement Division
Material Measurement Laboratory

Johanna Camara
Chemical Sciences Division
Material Measurement Laboratory

Elena S.C. Wood
Chemical Sciences Division
Material Measurement Laboratory

N. Alan Heckert
Statistical Engineering Division
Information Technology Laboratory

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NIST Author ORCID IDs
Ashley Beasley-Green: 0000-0002-2065-4218
Johanna Camara: 0000-0002-9415-8452
Elena S.C. Wood: 0009-0000-8537-3006
N. Alan Heckert: 0000-0002-8430-6757

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

Abstract

The National Institute of Standards and Technology (NIST) Standard Reference Material® (SRM®) 3666 Albumin and Creatinine in Frozen Human Urine is a secondary measurement standard for albumin and creatinine in human urine. The material is intended for use in a calibration hierarchy, in the validation of measurement procedures, or as quality control material for the determination of albumin, creatinine, or the albumin to creatinine ratio (ACR) in human urine. A unit of SRM 3666 consists of four (4) vials of frozen pooled human urine with four (4) different levels (Level I to Level IV) of endogenous albumin determined using the NIST reference measurement procedure (RMP) for albumin in urine, and creatinine determined using a modification of the NIST RMP for creatinine in serum. The certified values and associated uncertainties for albumin and creatinine were used to calculate the ACR for each level (Level I to Level IV). This publication documents the production, measurement processes, results, and statistical evaluations involved in the production and certification of SRM 3666.

Keywords

Albumin, Albumin-to-Creatinine Ratio (ACR), Creatinine, Human Urine, Reference Measurement Procedure (RMP), Standard Reference Material (SRM).

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EXECUTIVE SUMMARY

Patient care decisions made by healthcare practitioners for disease diagnosis and management are influenced by the validity of clinical laboratory results. Uniformity of clinical laboratory results is essential for healthcare practitioners to provide accurate and consistent patient care. When clinical results are standardized, the clinical value is precise, equivalent, and independent of method or laboratory. Equivalent clinical results can be achieved by establishing metrological traceability of the results to higher-order reference materials and measurement procedures. However, when clinical results are not standardized, meaning a different value may be obtained for the same clinical sample using different methods or clinical laboratories, the entire spectrum of patient care can be affected, from the delivery of erroneous medical decisions to inflated healthcare costs. Therefore, metrological traceability is applied to establish a traceability framework that underpins the confidence and global comparability of clinical results used in the diagnosis and management of disease.

ACKNOWLEDGEMENTS

The authors thank Dr. Greg Miller and Dr. Lorin Bachmann of the Virginia Commonwealth University for conducting the preliminary assessments using the routine clinical assays for albumin and creatinine, the National Institute of Diabetes and Digestive and Kidney Diseases Laboratory Working Group, and the International Federation of Clinical Chemistry Working Group for the Standardization of Albumin Assays in Urine.

1. INTRODUCTION

Kidney disease is a major global public health issue, with chronic kidney disease (CKD) representing one of the most prominent causes of death worldwide (12th leading cause of death in 2017). It was estimated in 2017 that more than 10 % of the general global population was diagnosed with CKD, which totals to greater than 800 million individuals [1]. Based on data from the National Health and Nutrition Examination Survey (NHANES; 2017 to 2020), an estimated 15 % of adults in the United States (37 million individuals) have been diagnosed with CKD [2,3]. The public health and economic impact of CKD and other renal diseases have led to the need for the accurate detection of kidney disease biomarkers, such as urine albumin, for early diagnosis, evaluation of treatment efficacy, and disease management.

Urine albumin is a major diagnostic and prognostic biomarker of renal disease and is used for clinical decisions associated with renal therapy. Due to the clinical importance of urine albumin, accurate and precise measurement is key for the early detection of renal dysfunction and evaluation of treatment efficacy. Normal excretion of protein in urine is less than 150 mg/L per 24 h and normal urine is composed of approximately 10 mg/L of albumin [4]. Due to the abundance of albumin in human plasma and subsequent presence in human urine, albumin has become a key protein used in the assessment of urinary excretion of plasma proteins. Albumin is a globular protein produced in the liver and functions as a transport protein in plasma and a regulator of plasma oncotic pressure [5]. Mature, native albumin contains 585 amino acids (removal of signal sequence amino acids 1 to 24) arranged into three distinct domains (Fig. 1) [5]. Normal urine albumin levels range from 0 mg/L to 30 mg/L (normoalbuminuria); however, increased excretion of albumin in urine is divided into two groups: microalbuminuria (30 mg/L to 300 mg/L) and macroalbuminuria (> 300 mg/L) [6]. To selectively measure albumin in urine, current clinical methodologies utilize affinity-based techniques, such as enzyme-linked immunosorbent assays (ELISAs) and immunoturbidity assays [7,8]. These affinity-based methods are routinely used in clinical laboratories to detect albumin in urine; however, there are distinct measurement challenges that affect the accuracy and precision of clinical results. Currently, no reference material for urine albumin exists to support clinical methods. Most clinical urine albumin results are traceable to ERM-DA470 (Institute for Reference Materials and Measurements; Geel, Belgium), a higher-order serum protein reference material with an assigned concentration value of 39.7 g/L (39,700 mg/L) for albumin, which is over 1000-fold higher than the clinical range for microalbuminuria (30 mg/L to 300 mg/L) [6, 9-11].

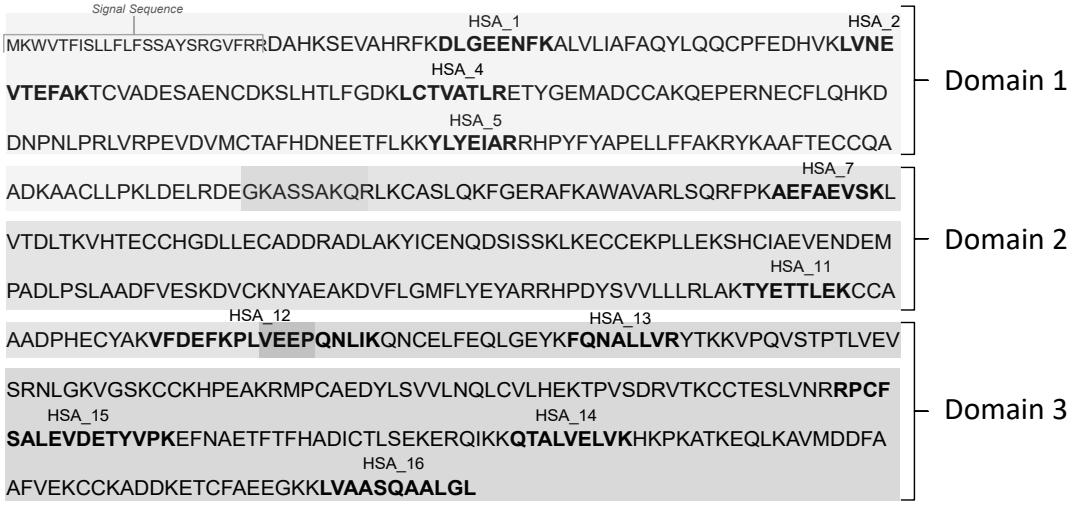

Fig. 1. Amino acid sequence of albumin (human serum albumin, HSA) illustrating the 23 MRM transitions used in NIST RMP [13-15] in bold and the three (3) domains (I, II, and III).

To support the accuracy and comparability of clinical urine albumin measurements, NIST has partnered with the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Laboratory Working Group (LWG) and the International Federation of Clinical Chemistry (IFCC) Working Group for the Standardization of Albumin Assays in Urine (WG-SAU) to develop a reference measurement system for urine albumin. A reference measurement system, as defined in ISO 17511, is a "*measuring system accepted as fit for its intended purpose in assessing or establishing measurement trueness for quantity values obtained from other MPs for the measurand; comprised of: 1) a unit of measurement, 2) a definition of the measurand, 3) RMP(s), 4) RM(s), and 5) one or more laboratories providing reference measurement services*" [12]. The reference materials and measurement procedures in the urine albumin reference measurement system will establish an unbroken chain that links routine clinical results to the International System of Units (SI) (Fig. 2) [12]. Figure 2 illustrates the reference materials and measurement procedure developed by NIST in the calibration hierarchy to support clinical urine albumin measurements [12]. NIST has developed an RMP [13-15] for the detection of albumin in human urine and a series of higher-order reference materials, SRM 2925 Recombinant Human Serum Albumin Solution (Primary Reference Calibrator for Urine Albumin, Frozen) [16,17] and SRM 3666 Albumin and Creatinine in Frozen Human Urine. The NIST RMP for Urine Albumin is a targeted, multiplexed procedure that incorporates isotope dilution-liquid chromatography-tandem mass spectrometry (ID-LC-MS/MS), SRM 2925 (unlabeled calibrant), and a full-length isotopically labeled (^{15}N) internal standard (IS) for absolute quantification of albumin in urine [13-15]. The NIST RMP for Urine Albumin and validation attributes are detailed in Ref. [13-15]. The RMP is intended for use in the value assignment of albumin in SRM 3666. SRM 2925 is a highly pure solution of recombinant human serum albumin (HSA) and is intended for use in the calibration of LC-MS/MS procedures for the determination of albumin in urine [16,17]. SRM 2925 serves as a primary reference material for the urine albumin reference measurement system (Fig. 2) and is currently listed in the Joint Committee for Traceability in Laboratory Medicine (JCTLM) database for reference materials [18]. The JCTLM database is an internationally recognized database of higher-order reference materials for clinical applications, and the inclusion of SRM

2925 [19] and the RMP for Urine Albumin [20] in the database is an initial step in establishing a reference measurement system for urine albumin to support global comparability of clinical results [18]. SRM 3666 is a four (4)-level (Level I to Level IV) human urine material intended for use as a secondary reference material to support the accuracy and comparability of clinical urine albumin and urine creatinine results used in clinical decisions for kidney disease. The material is intended for use in a calibration hierarchy, in the validation of measurement procedures, or as quality control material for the determination of albumin, creatinine, or the albumin-to-creatinine ratio (ACR) in human urine. This document outlines the production, value-assignment, characterization, and statistical evaluations of SRM 3666.

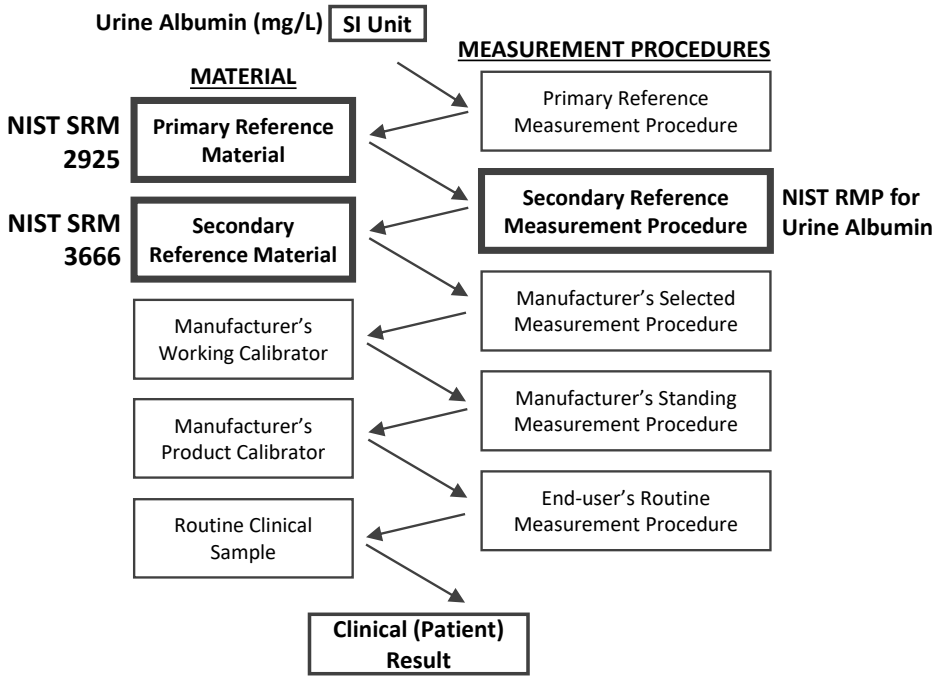

Fig. 2. The proposed full metrological traceability framework for clinical urine albumin results using NIST SRMs and RMP [Adapted from ISO 17511, Ref. 12]

2. PRODUCTION OF SRM 3666

An open solicitation for quotations was issued for the acquisition and pooling/packaging of the human urine material used for preparation of SRM 3666 (Level I to Level IV). The following Sections summarize the scope for the material acquisition and pooling/packaging contracts.

2.1. Acquisition of Human Urine Material for SRM 3666

The material acquisition contract describes the requirements for collection of fresh, non-frozen single donor human urine specimens with target endogenous urine albumin levels within the concentration ranges and quantities outlined in Table 1. The material acquisition contract (Contract# SB134117SE0320) was awarded to Virginia Commonwealth University (VCU).

Table 1. Target endogenous albumin ranges and quantities at each level (Level I to Level IV)

SRM 3666 Level	Target Endogenous Albumin Ranges (mg/L)	Vial Cap Color	Volume of Total Human Urine (mL)	Total Number of Specimen Donors	Volume of Urine per Specimen Donor (mL)
Level I	5 mg/L to 10 mg/L	Purple	2700	54	50
Level II	20 mg/L to 50 mg/L	Yellow	2700	54	50
Level III	60 mg/L to 180 mg/L	Blue	2700	54	50
Level IV	200 mg/L to 600 mg/L	Red	2700	54	50

The fresh, individually packaged single donor human urine specimens were stored at ≤ -70 ºC and the frozen specimens (in batches, with a minimum of 12 shipments) were shipped overnight on dry-ice to a NIST-specified institution for further processing (pooling and packaging). There were no donor specifications regarding age, gender, ethnicity, body mass index (BMI), or health status for the specimen collection process. All single donor urine specimens with a positive result for: urine nitrites, leukocyte esterase, or the presence of blood in urine using an FDA-approved urinalysis dipstick diagnostic assay were excluded. Through visual inspection, urine specimens with a color other than pale yellow, yellow, or amber were also excluded. The fresh urine specimens were stored in the original sterile collection containers at room (ambient) temperature (20 ºC to 25 ºC) for no more than 6 h after collection. Following collection, the urine specimen was centrifuged at 2000 × g_n for 10 min at low temperature (2 ºC to 5 ºC). The urine supernatant was transferred to one or more 50 mL sterile polypropylene containers and stored at ≤ -70 ºC. To ensure the urine specimen possessed endogenous urine albumin within the target clinical intervals listed in Table 1, a routine clinical assay for urine albumin was used to measure the endogenous urine albumin content in each urine specimen. No albumin was spiked in, nor were the high endogenous urine albumin levels diluted to achieve the target urine albumin concentrations. Each single donor urine specimen was labeled with the following information: a donor code, container number (if multiple containers per donor), and the urine albumin level (Level I to Level IV).

2.2. Pooling and Packaging of SRM 3666

The material pooling and packaging contract outlines the requirements for pooling, processing, and vialing of the multi-level (Level I to Level IV) human urine material. The material pooling and packaging contract (Contract# SB134118SE0090) was awarded to Solomon Park. The frozen single donor human urine specimens were shipped to Solomon Park by VCU. Prior to processing, the frozen material was thawed at 20 ºC to 25 ºC (room temperature) for a maximum of 2 h and each level of SRM 3666 was processed separately. The thawed material was mixed with gentle inversion (10 cycles) and centrifuged at 2000 × g_n for 10 min at low temperature (2 ºC to 8 ºC). The single donor specimens were combined according to the composition specified by NIST to yield a total volume of 2.7 L for each level. The pooled bulk material was mixed (gentle) overnight (12 h to 18 h, not to exceed 18 h) at 2 ºC to 5 ºC to allow any precipitates or aggregates to form. After mixing, the pooled bulk material was placed on a sterile 0.2 µm polyvinylidene fluoride (PDVF; low-protein binding membrane filter) filtration device at 2 ºC to 5 ºC. Approximately 1 mL (± 0.05 mL) of filtered bulk pooled material was aliquotted into 1 mL sterile polypropylene screw cap vials at a temperature between 2 ºC to 5 ºC. A different vial cap color was used for each level (Level I to Level IV) to visually differentiate the four levels (Table 1). The frozen vials of SRM 3666 (Level I to Level IV) were packaged and shipped to NIST.

2.3. Preliminary Assessment of Packaged Human Urine Material

To evaluate the suitability of the packaged human urine material for use as SRM 3666, preliminary measurements of the albumin and creatinine content were conducted by NIST and VCU (through a collaboration). A total of twelve (12) vials of SRM 3666 were selected for the preliminary assessment, which represents three (3) vials selected from each level (Level I to Level IV) spanning the entire lot. The preliminary assessment sampling plan is outlined in Appendix A, Table A1. A minimum of two (2) technical replicates per vial were performed for each method.

To confirm the endogenous albumin levels for the packaged human urine material were within the target ranges (outlined in Table 1), the endogenous urine albumin content was assessed using a routine clinical microalbumin assay (Abbott Architect Microalbumin Assay) by VCU. The Abbott Architect Microalbumin Assay is a turbidimetric immunoassay that utilizes an anti-human albumin polyclonal antibody to detect albumin in human urine. The polyclonal antibody-albumin complex forms an insoluble aggregate that increases the turbidity of the solution. The degree of turbidity is proportional to the concentration of albumin in the urine specimen and is measured optically on the Abbott Architect c8000 instrument. The endogenous urine creatinine content was assessed via a routine clinical creatinine assay (Abbott Architect Enzymatic Creatinine Assay) by VCU. The Abbott Architect Enzymatic Creatinine Assay is a colorimetric method that is based on enzymatic principle to determine creatinine content in human urine. Creatinine in the sample is hydrolyzed by creatininase to form creatine and creatine is hydrolyzed by creatinase to form sarcosine and urea. The sarcosine produced from this reaction is then oxidized by sarcosine oxidase to produce glycine, formaldehyde, and hydrogen peroxide. The hydrogen peroxide produced from this reaction reacts with 4-aminoantipyrine and N-ethyl-N-sulfopropyl-m-toluidine in the presence of peroxidase to yield a quinoneimine dye. The change in absorbance is directly proportional to the concentration of creatinine in the sample. In addition to the routine clinical assays, the NIST RMP for Urine Albumin [13-15,20] and NIST modified-RMP for Serum Creatinine [21,22] were also used in the preliminary assessment of the packaged human urine material. The preliminary assessment results of the human urine material from the routine clinical assays (VCU) and the MS-based methods (NIST) are listed in Appendix A, Tables A2 and A3. Preliminary assessment of the endogenous albumin content of the human urine material supports the use of the material as SRM 3666.

3. CERTIFICATION MEASUREMENTS OF SRM 3666

3.1. Density of SRM 3666

Density values were needed for unit conversion (mass fraction to mass concentration) of the certified values and were determined by the Lang-Levy pipet method [23]. A 500 µL Lang-Levy pipet was calibrated with water at ambient room temperature (19 ºC). The water weighing was performed in four (4) replicates and the density of water at 19 ºC ($\rho_{H_2O,19\,℃}$) was determined using [24]:

$$\rho_{H_2O,19\,℃} = \frac{(999.84847+6.337563\times10^{-2}t-8.523829\times10^{-3}t^2+6.943248\times10^{-5}t^3-3.821216\times10^{-7}t^4)}{1000}, \quad (1)$$

where t is the observed temperature (19 ºC) at which the water mass was measured. The volume (mL) of the pipet was determined from the water sample using:

$$V_{H_2O,19\,℃} = \frac{M_{H_2O,19\,℃}}{\rho_{H_2O,19\,℃}}, \quad (2)$$

where $M_{H_2O,19\,℃}$ is the mass of water at the observed temperature (19 ºC). The volume calibration measurements of the nominal 500 µL Lang-Levy pipette at 19 ºC are shown in Appendix B, Table B1. The volume at 19 ºC was determined to be 0.49976 mL ± 0.00092 mL (mean ± standard deviation).

To assess the density of SRM 3666, four (4) vials were randomly selected, thawed at room temperature (19 ºC), and pooled for each level (sampling plan listed in Appendix B, Table B2). The weighing procedure was repeated for each urine pool in replicate (4). The density results for SRM 3666 (Level I to Level IV) were calculated using:

$$\rho_{SRM\ 3666} = \frac{M_{SRM\ 3666,19\,℃}}{V_{H_2O,19\,℃}}. \quad (3)$$

The certified density values for SRM 3666 (Level I to Level IV) are shown in Table 2. Statistical analysis of the data for the determination of density for SRM 3666 was provided by the NIST Statistical Engineering Division (SED).

Table 2. Certified Human Urine Density of SRM 3666 at 19 °C

Material Level	Measurand	Density [a] (g/mL)
Level I	Human Urine Density at 19 °C	1.01519 ± 0.00058
Level II	Human Urine Density at 19 °C	1.01397 ± 0.00048
Level III	Human Urine Density at 19 °C	1.01784 ± 0.00058
Level IV	Human Urine Density at 19 °C	1.01549 ± 0.00070

[a]Values are expressed as $x \pm U(x)$, where x is the certified value and $U(x)$ is the expanded uncertainty of the certified value. The expanded uncertainty is calculated as $U(x) = ku_c$, where u_c is the combined uncertainty, and k is the coverage factor. For the certified values shown in Table 2, $k = 2$. The true value of the analyte is believed to lie within the interval $x \pm U(x)$. To propagate this uncertainty, treat the certified value as a normally distributed random variable with mean x and standard deviation $U(x)/2$.

3.2. Value-Assignment of Urine Albumin

The value assignment measurements for albumin in SRM 3666 (Level I to Level IV) detailed in this Section were performed using the NIST RMP for albumin determination in human urine by ID-LC-MS/MS [13-15,20]. Certification of the albumin concentrations in SRM 3666 was performed at NIST, and the statistical analysis of the data was provided by NIST SED.

3.2.1. Materials

NIST SRM 2925 Recombinant Human Serum Albumin Solution (Primary Reference Calibrator for Urine Albumin) (Frozen) [16-17,19]; ^{15}N-labeled recombinant human serum albumin (*r*HSA) (Albumin Biosciences, Huntsville, AL); ammonium bicarbonate (AMBIC); Trypsin-Gold MS-grade (Promega, Madison, WI, USA); dithiothreitol (DTT, Pierce); iodoacetamide (IAM, Pierce); high-purity LC-MS grade water/0.1 % (volume fraction) formic acid and acetonitrile/0.1 % (volume fraction) formic acid (Honeywell Burdick and Jackson).

3.2.2. Certification Sampling Plan

A total of twelve (12) vials per level of SRM 3666 were randomly selected from the lot via a stratified random sampling scheme. See Appendix C, Table C1 for the certification sampling plan.

3.2.3. Certification Measurement Process

The NIST RMP incorporates a full-length ^{15}N-labeled IS to selectively target and quantify full-length albumin in human urine with a high degree of accuracy and precision [13-15,20]. The MRM (multiple reaction monitoring) chromatographic and MRM retention time (RT) profiles (Appendix C, Fig. C1 and C2) of the SRM 3666 samples (unlabeled, endogenous albumin) and the IS (^{15}N-labeled *r*HSA) were consistent across all four levels (Level I to Level IV). Performance of the IS was also consistent across the 23 MRM transitions of each level (Level I to Level IV), as shown in the normalized IS peak area plot Appendix C, Fig. C3. Five (5) 3-point calibration curves, representing the 5 *qt*-MRM transitions, were generated using the concentration and peak area ratios of the calibration solutions for each level of SRM 3666 (Level I to Level IV) (Appendix C, Fig. C4).

Consensus means analysis of the albumin mass fraction (mg/g) results for the *qt*-MRM transitions was conducted (n = 160; 5 MRM transitions, twelve (12) vials and one (1) process replicate) for each level (Level I to Level IV) to determine the overall endogenous albumin content in each level of SRM 3666. The certified density values of SRM 3666 (Level I to Level IV) (Table 2) were used for the unit conversion from mass fraction (mg/g) to mass concentration (mg/L). The consensus mass fraction values ($\bar{\bar{X}}_{mg/g}$) and mass concentration values ($\bar{\bar{X}}_{mg/L}$) for albumin in SRM 3666 (Level I to Level IV) are shown in Table 3 [13-15, 20].

The certified albumin mass concentration values for SRM 3666 are consistent with the expected target clinical ranges and the preliminary results obtained via the routine clinical assay and the NIST RMP [13-15, 20], as shown in Appendix C, Table C2.

Table 3. Certified Values for Albumin in SRM 3666

Material Level	Mass Fraction (mg/g) (Mean ± Expanded Uncertainty[a])	Mass Concentration[b] (mg/L) (Mean ± Expanded Uncertainty[a])
Level I	0.008 ± 0.001	8.28 ± 1.12
Level II	0.031 ± 0.003	31.11 ± 2.64
Level III	0.111 ± 0.011	112.77 ± 10.79
Level IV	0.355 ± 0.031	360.50 ± 31.11

[a]Values are expressed as $x \pm U(x)$, where x is the certified value and $U(x)$ is the expanded uncertainty of the certified value. The expanded uncertainty is calculated as $U(x) = ku_c$, where u_c is the combined uncertainty, and k is the coverage factor. For calculation of the combined uncertainty for albumin certified values see Ref. 20. For the certified values shown in Table 2, $k = 2$. The true value of the analyte is believed to lie within the interval $x \pm U(x)$. To propagate this uncertainty, treat the certified value as a normally distributed random variable with mean x and standard deviation $U(x)/2$.
[b]Mass concentration values were calculated from mass fraction values using the measured human urine density for each level, listed in Table 2.

3.2.4. Calculation of Uncertainty for Urine Albumin

The uncertainty components associated with the NIST RMP [13-15, 20] are combined to derive $\bar{\bar{X}}_{mg/g}$ combined uncertainty ($u_{mg/g}$) using [14,15]:

$$u_{mg/g} = \sqrt{u^2{}_{Type\ A} + u^2{}_{Type\ B}}\,. \tag{4}$$

where $u_{Type\ A}$ represents the Type A uncertainty of $\bar{\bar{X}}_{mg/g}$ determined via the DSL-HHD or DSL-bootstrap methods and the $u_{Type\ B}$ represents the combined Type B uncertainties ($u_{SRM\ 2925}$, u_{Mass}, u_{PAR}) [14,15]. The u_{PAR} represents the combined uncertainty for $u_{PAR,Type\ A}$ and $\bar{u}_{ccur}$ for the 5 *qt*-MRM transitions [14,15]:

$$u_{PAR} = \sqrt{u^2{}_{PAR,Type\ A} + \bar{u}^2{}_{ccur}}\,, \tag{5}$$

where $\bar{u}_{ccur}$ represents the mean calibration curve uncertainty ($\bar{u}_{ccur}$) of the 5 *qt*-MRM transitions [14,15]. The final combined standard uncertainty ($u_{mg/L}$) of the consensus mass concentration value ($\bar{\bar{X}}_{mg/L}$) for the 5 *qt*-MRM transitions is calculated using [14,15]:

$$u_{mg/L} = \sqrt{d^2 u^2{}_{mg/g} + \bar{\bar{X}}_{mg/g} u^2{}_d}\,, \tag{6}$$

where d represents the density of SRM 3666 (Level I to Level IV) and u_d represents the combined uncertainty of the density value (Table 2) [14,15]. The $u_{mg/g}$ and $u_{mg/L}$ values are expressed as expanded uncertainties (U), which are obtained by multiplying the combined standard uncertainty ($u_{mg/g}$ or $u_{mg/L}$) by a coverage factor (k) [14,15]:

$$U_{mg/g} = k \times u_{mg/g} \qquad \text{or} \qquad U_{mg/L} = k \times u_{mg/L}, \tag{7}$$

where $k = 2$ is used for calculation of the expanded uncertainty (approximately 95 % confidence level) of urine albumin via the NIST RMP [13-15, 20].

3.3. Value-Assignment of Urine Creatinine

The value assignment measurements for creatinine in SRM 3666, detailed in this Section, were performed using the NIST RMP for Serum Creatinine, an isotope dilution-liquid chromatography-mass spectrometry (ID-LC-MS) method, modified for creatinine in urine [21,22]. Certification of the creatinine concentrations in SRM 3666 (Level I to Level IV) was performed at NIST, and the statistical analysis of the data for the determination of creatinine in SRM 3666 was provided by NIST SED.

3.3.1. Materials

NIST SRM 914b Creatinine (mass purity of 99.9 % ± 0.1 %, Certificate of Analysis); stable isotope labeled IS material (*d3*-creatinine, Cayman Chemical Company); NIST SRM 3667 (quality control material); all calibration solutions, urine sample dilutions, and mobile phase were prepared using HPLC-grade water from Burdick & Jackson; HPLC-grade ammonium acetate (Fisher Scientific); and Hydrochloric (HCl) acid solution (2 mol/L, Fluka).

3.3.2. Certification Sampling Plan

See Appendix C, Table C1 for the certification sampling plan.

3.3.3. Certification Measurement Process

Calibration solutions, which contained known unlabeled:labeled creatinine mass ratios, were gravimetrically prepared. The IS solutions were added to all samples (SRM 3666 and SRM 3667) at the beginning of sample preparation process. The processed samples (calibration solutions, SRM 3667 quality control solutions, and SRM 3666 samples) were analyzed via liquid chromatography-mass spectrometry (LC-MS) and the unlabeled:labeled creatinine peak area ratios of the SRM 3667 and SRM 3666 samples were converted to mass ratios using data from the calibration curves generated from the calibration solutions. The mass ratios were then solved for the mass of the unlabeled creatinine, and the concentration of unlabeled creatinine in each sample was calculated.

Four (4) stock solutions of creatinine were gravimetrically prepared with mass fractions of ≈ 44 µg/g to 87 µg/g and the IS solution was gravimetrically prepared with an estimated mass fraction of ≈ 100 µg/g. From the stock solutions, working solutions were gravimetrically prepared, containing both unlabeled creatinine and IS. Calibration solution mixes were then diluted to a final volume of 1 mL with water, this resulted in calibrants containing ≈ 4 µg to 6 µg of creatinine and ≈ 5 µg of d_3-creatinine. Prior to processing, the SRM 3666 samples and quality control samples (SRM 3667) were thawed at room temperature (19 °C) for 1 h. The SRM 3667 control urine samples were prepared in duplicate from one vial on each of four days of analysis and the

SRM 3666 urine samples were prepared in duplicate from twelve vials of each of four levels. The twelve (12) vials of SRM 3666 for each level were equally distributed over four separate preparation and analysis runs. All LC-MS analyses were performed on an Agilent 1200 Series LC system with an Agilent 6130 Quadrupole LC-MS. The column utilized was a Phenomenex (Torrance, CA) Luna C18(2), 25 cm × 4.6 mm, 5 µm particle. Selected ion monitoring (SIM) was used to detect creatinine at *m/z* 114 and d_3-creatinine at *m/z* 117. An IS approach and relative response factor (RRF) were used for calibration according to the equation [21,22]:

$$RRF = \frac{(A_{Creatinine,Cal} \times M_{IS,Cal})}{(A_{IS,Cal} \times M_{Creatinine,Cal} \times P_{creatinine})}, \quad (8)$$

where $A_{Creatinine,Cal}$ represents the peak area for creatinine (unlabeled) for the calibration solution, $M_{IS,Cal}$ represents the mass of the IS in the calibration solution, $A_{IS,Cal}$ represents the peak area for the IS in the calibration solution, $M_{Creatinine,Cal}$ represents the mass of creatinine (unlabeled) in the calibration solution, and $P_{creatinine}$ represents purity correction factor for creatinine. The mean RRF values for the LC-MS runs were 1.142 (set 1), 1.354 (set 2), 1.154 (set 3), and 1.145 (set 4). Example chromatograms of SRM 3666 Level I to Level IV are shown in Appendix D, Fig. D1-D4, respectively.

The RRF was averaged from duplicate injections of each of four (4) independently prepared calibration solutions over a narrow-bracketed mass ratio range (0.8 to 1.2 mass ratio, creatinine: d_3-creatinine). The RRF was applied to samples to determine the mass fraction ($x_{\mu g/g}$) of creatinine according to the equation [21,22]:

$$x_{\mu g/g} = \frac{(A_{Creatinine,S} \times M_{IS,S})}{(A_{IS,S} \times M_S \times RRF)}, \quad (9)$$

where $A_{Creatinine,S}$ represents the peak area for creatinine (unlabeled) in the sample, $M_{IS,S}$ represents the mass of the IS in the sample, $A_{IS,S}$ represents the peak area for the IS in the sample, and M_S represents the mass of creatinine (unlabeled) in the sample. The consensus mass fraction value ($\bar{\bar{x}}_{\mu g/g}$) is calculated by combining the $x_{\mu g/g}$values for creatinine using consensus mean analysis via the DSL model (random-effects model):

$$y_{ij} = \mu + m_i + \varepsilon_{ij} \quad (10)$$
$$i = 1, 2 \ldots, n_{mm}; j = 1,2, \ldots, n_i,$$

where i indexes the measurements, j indexes replications within the method, n_{mm} represents the number of measurements, n_i represents replications within the method, μ represents the grand mean for all measurements, m_i represents the mean of the measurements (relative to grand mean), and ε_{ij} represents variance between the measurements. The $\bar{\bar{x}}_{\mu g/g}$ and the density of SRM 3666 (Level I to Level IV) (Table 2) are used to derive the consensus mass concentration ($\bar{\bar{x}}_{mg/dL}$) according to the equation:

$$\bar{\bar{x}}_{mg/dL} = \frac{(\bar{\bar{x}}_{\mu g/g} \times d_{urine})}{10}. \quad (11)$$

where d_{urine} represents the density of the urine sample (SRM 3666). The urine creatinine $\bar{\bar{x}}_{\mu g/g}$ and $\bar{\bar{x}}_{mg/dL}$ and associated uncertainties (consensus mean ± expanded uncertainty) for Level I to Level IV are listed in Table 4. The creatinine values measured by the modified-NIST RMP [21,22] are consistent with the preliminary assessment values provided by the routine clinical assay (Jaffe, VCU) (Appendix D, Table D1).

Table 4. Certified Values for Creatinine in SRM 3666

Material Level	Mass Fraction (µg/g) (Mean ± Expanded Uncertainty[a])	Mass Concentration[b] (mg/dL) (Mean ± Expanded Uncertainty[a])
Level I	1178.96 ± 25.05	119.69 ± 2.63
Level II	1209.58 ± 26.28	122.65 ± 2.73
Level III	1249.58 ± 26.37	127.19 ± 2.78
Level IV	1289.01 ± 27.71	130.90 ± 2.96

[a]Values are expressed as $x \pm U(x)$, where x is the certified value and $U(x)$ is the expanded uncertainty of the certified value. The expanded uncertainty is calculated as $U(x) = ku_c$, where u_c is the combined uncertainty, and k is the coverage factor. For the certified values shown in Table 2, $k = 2.201$. The true value of the analyte is believed to lie within the interval $x \pm U(x)$. To propagate this uncertainty, treat the certified value as a normally distributed random variable with mean x and standard deviation $U(x)/2.201$.
[b]Mass concentration values were calculated from mass fraction values using the measured human urine density for each level, listed in Table 2.

3.3.4. Calculation of Uncertainty for Urine Creatinine

The uncertainty components associated with the NIST RMP for Serum Creatinine, modified for creatinine in urine [21,22], are combined to derive the consensus mass fraction ($\bar{\bar{x}}_{\mu g/g}$) combined uncertainty ($u_{mg/g}$) using:

$$u_{\mu g/g} = \sqrt{u^2{}_{Type\ A} + \sum_{i=1}^{2} u^2{}_{Type\ B}}, \tag{12}$$

where $u_{Type\ A}$ represents the Type A uncertainty of consensus mass fraction value ($\bar{\bar{x}}_{\mu g/g}$) determined via the DSL-HHD or DSL-bootstrap methods and the $u_{Type\ B}$ represents the combined Type B uncertainties (0.3 % for the purity of NIST SRM 914b and 1 % for the possible undetected inferences) [21,22]. The final combined standard uncertainty ($u_{mg/dL}$) of the consensus mass concentration value (mg/dL) is calculated using:

$$u_{mg/dL} = \sqrt{d^2 u^2{}_{\mu g/g} + \bar{\bar{x}}_{\mu g/g} u^2{}_{d}}, \tag{13}$$

where d represents the density of SRM 3666 (Level I to Level IV) and u_d represents the combined uncertainty of the density value (Table 2). The $u_{\mu g/g}$ and $u_{mg/dL}$ values are expressed as expanded uncertainties (U), which are obtained by multiplying the combined standard uncertainty ($u_{\mu g/g}$ or $u_{mg/dL}$) by a coverage factor (k):

$$U_{\mu g/g} = k \times u_{\mu g/g} \qquad \text{or} \qquad U_{mg/dL} = k \times u_{mg/dL}. \tag{14}$$

where $k = 2.201$ is used for calculation of the expanded uncertainty (approximately 95 % confidence level) of urine creatinine via the modified-NIST RMP [21,22].

3.4. Value-Assignment of Albumin-to-Creatinine Ratio (ACR)

The value assignment results for albumin-to-creatinine ratio (ACR) in SRM 3666 (Level I to Level IV), detailed in this Section, were determined from the certified mass concentration values and associated uncertainties of both albumin (Table 3) and creatinine (Table 4). Statistical analysis of the data for the determination of ACR in SRM 3666 was provided by NIST SED.

3.4.1. Certification Measurement Process

The ACR certified value for each level was determined from a ratio of the certified mass concentration values for albumin to creatinine using the following:

$$ACR = \frac{A}{C}, \tag{15}$$

where *A* represents the certified mass concentration value for albumin and *C* represents the certified mass concentration value for creatinine.

To determine the uncertainty of the ACR certified value, the propagation of uncertainty (also called propagation of error) was applied. Propagation of uncertainty is a technique for determining the approximate uncertainty of a function of two or more variables where the uncertainties of the individual variables are known [26-29]. Propagation of uncertainty formulas have been determined for a number of commonly used functions, including the ratio of two variables [26-29]. The certified values for albumin and creatinine were determined using separate measurement procedures and samples. Therefore, they can be considered independent and the covariance terms in the propagation of uncertainty formula can be ignored. The resulting propagation of uncertainty formula used to determine the standard uncertainty of the certified ACR value is:

$$u_{ACR} = \left|\frac{A}{C}\right| \times \sqrt{\left(\frac{u_A}{A}\right)^2 + \left(\frac{u_C}{C}\right)^2}. \tag{16}$$

where *A* represents the certified value for albumin (mass concentration, mg/L), u_A represents the combined standard uncertainty for albumin, *C* represents the certified value for creatinine (mass concentration, mg/dL) , and u_C represents the combined standard uncertainty for creatinine. The ACR (mg/g) certified values and associated expanded uncertainties ($k = 2$) for SRM 3666 (Level I to Level IV) are listed in Table 5.

Table 5. Certified Values for ACR in SRM 3666

Material Level	Value[b] (mg/g) (Mean ± Expanded Uncertainty[a])
Level I	6.92 ± 0.95
Level II	25.36 ± 2.22
Level III	88.66 ± 8.70
Level IV	275.40 ± 24.57

[a]Values are expressed as $x \pm U(x)$, where x is the certified value and $U(x)$ is the expanded uncertainty of the certified value. The expanded uncertainty is calculated as $U(x) = ku_c$, where u_c is the combined uncertainty, and k is the coverage factor. For the certified values shown in Table 2, $k = 2$. The true value of the analyte is believed to lie within the interval $x \pm U(x)$. To propagate this uncertainty, treat the certified value as a normally distributed random variable with mean x and standard deviation $U(x)/2$.
[b]The albumin-to-creatinine ratio (ACR) is based on the combination of the certified values for albumin (Table 3) and creatinine (Table 4).

4. HOMOGENEITY OF SRM 3666

The homogeneity assessment of the material (albumin and creatinine) was conducted in parallel with the certification analyses. A stratified sampling plan was devised to evaluate homogeneity across the lot. The sample size used for the homogeneity assessment was 130 µL for albumin and 50 µL for creatinine. There was no apparent trend in the data when plotted against the sequence in which the vials were filled. The homogeneity assessment results are shown in Appendix E.

5. STABILITY OF SRM 3666

Stability for urine albumin and creatinine was evaluated following the certification analyses. The stability assessment results are shown in Appendix F.

For the urine albumin short-term (7-day) stability study, a random sampling scheme was used to assess the degree of albumin degradation under potential temperature conditions encountered during shipment from NIST to the end user. The measured values for urine albumin were within the confidence interval of the certified value ($\overline{\overline{X}}_{mg/L} \pm U_{mg/L}$), indicating no significant degradation. Furthermore, no observable trends were detected in the data, suggesting that routine shipping temperatures will not affect the material's integrity over a 7-day period.

For the urine creatinine long-term stability study, measurements were acquired using the modified-NIST RMP [21,22] to assess the validity of the certification value under the storage conditions [30,31]. The measured values for urine creatinine were within the confidence interval of the certified value ($\bar{\bar{x}}_{mg/dL} \pm U_{mg/dL}$), indicating no significant degradation over a 2-year period. Therefore, the material is considered stable under the specified storage conditions and remains fit for its intended use.

6. COMMUTABILITY OF SRM 3666

The commutability assessment of urine albumin and creatinine in NIST SRM 3666 (Level I to Level IV) was conducted by analyzing single-donor human urine clinical samples and SRM 3666 using the NIST RMP for Urine Albumin [13-15, 20], the modified-NIST RMP for Serum Creatinine [21,22], and routine in vitro diagnostic measurement procedures (IVD-MPs). The results indicated that urine albumin and creatinine in SRM 3666 (Level I to Level IV) showed satisfactory commutability for the evaluated IVD-MPs. This suggests that SRM 3666 is suitable for use as a secondary measurement standard in the calibration hierarchy for urine albumin and creatinine in select IVD-MPs. The commutability results are summarized in Appendix G, with a comprehensive description of the commutability assessment and findings provided in Ref. [30].

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APPENDIX

Appendix A. SRM 3666 – Preliminary Assessment

This section contains supplemental information to support the preliminary assessment of albumin and creatinine in SRM 3666 (Level I to Level IV).

Table A1. Sampling scheme for preliminary assessment of albumin and creatinine in SRM 3666. The vial selection was the same for each level (Level I to Level IV)

Method	Box #	Box Vial #	Lot Vial #
NIST RMP Urine Albumin and NIST modified-RMP for Serum Creatinine	1	10	10
	5	72	400
	11	30	850
	16	70	1300
	20	42	1600
	30	40	2418
	31	40	2500
Abbott Architect Microalbumin / Abbott Architect Enzymatic Creatinine Assays	1	50	50
	15	46	1180
	31	30	2460

Table A2. Preliminary assessment results of albumin in the packaged human urine material for use as SRM 3666 via the NIST RMP for Urine Albumin and a routine clinical immunoassay.

SRM 3666 Level	Target Endogenous Urine Albumin Content	Abbott Architect Microalbumin Assay (mg/L), n = 6 (VCU) (Mean ± Standard Deviation)	NIST RMP for Urine Albumin (ID-LC-MS/MS) (mg/L), n = 160 (Mean ± Standard Deviation)
Level I	5 mg/L to 10 mg/L	8.1 ± 0.2	9.1 ± 0.6
Level II	20 mg/L to 50 mg/L	34.4 ± 0.1	34.9 ± 1.5
Level III	60 mg/L to 180 mg/L	100.1 ± 0.9	105.6 ± 5.2
Level IV	200 mg/L to 600 mg/L	354.9 ± 3.5	344.2 ± 19.3

Table A3. Preliminary assessment results of creatinine in the packaged human urine material for use as SRM 3666 via the modified NIST RMP and a routine clinical assay (enzymatic).

SRM 3666 Level	Abbott Architect Enzymatic Creatinine Assay (mg/dL), n = 6 (VCU) (Mean ± Standard Deviation)	NIST RMP (ID-LC-MS) (mg/dL), n = 12 (Mean ± Standard Deviation)
Level I	116.7 ± 0.4	116.0 ± 0.3
Level II	118.4 ± 0.6	118.0 ± 0.8
Level III	123.0 ± 0.7	124.8 ± 0.6
Level IV	127.4 ± 0.4	129.0 ± 0.4

Appendix B. SRM 3666 – Density Determination

This section contains supplemental information to support density determination for SRM 3666 (Level I to Level IV).

Table B1. Calibration of Lang-Levy Pipet Volume with Water.

Water Sample	Mass (g)	Density at 19 °C (g/mL)	Volume at 19 °C (mL)	Mean Volume at 19 °C (mL) (n = 4) (Mean ± Standard Deviation)	%CV
Water-1	0.50029	0.9984	0.50109	0.49976 ± 0.00092	0.18
Water-2	0.49816	0.9984	0.49896		
Water-3	0.49875	0.9984	0.49955		
Water-4	0.49865	0.9984	0.49945		

Table B2. Sampling scheme for density (19 °C) determination of SRM 3666 (Level I to Level IV).

SRM 3666 Level	Sample Label	Box #	Vial #
Level I	**1**	3	17
	2	16	77
	3	22	36
	4	28	10
Level II	**1**	6	38
	2	9	81
	3	26	22
	4	29	27
Level III	**1**	6	7
	2	13	19
	3	19	72
	4	27	57
Level IV	**1**	2	60
	2	15	27
	3	23	11
	4	24	14

Appendix C. SRM 3666 – Certification of Albumin

This section contains supplemental information to support the certification of albumin in SRM 3666 (Level I to Level IV).

Table C1. Sampling scheme for certification of endogenous albumin and creatinine in SRM 3666. The vial selection was the same for each level (Level I to Level IV).

Measurand	Box #	Vial #1 Box Location #	Vial #1 Lot Number	Vial #2 Box Location #	Vial #2 Lot Number
Albumin	**5**	10	334	22	346
	11	53	863	80	890
	14	6	1059	56	1109
	19	21	1479	64	1522
	25	31	1975	74	2018
	30	17	2366	46	2395
Creatinine	**5**	11	335	23	347
	11	54	864	79	889
	14	7	1060	55	1108
	19	20	1478	65	1523
	25	32	1976	76	2020
	30	16	2365	45	2396

Table C2. Comparison of albumin results for the preliminary assessment and the material certification.

SRM 3666 Level	Target Endogenous Urine Albumin Content	**Certified Values** Mass Concentration of Albumin (mg/L) (NIST ID-LC-MS/MS Method), n = 280 (Mean ± Combined Standard Uncertainty)	**Preliminary Values** NIST RMP for Urine Albumin (ID-LC-MS/MS) (mg/L), n = 160 (Mean ± Standard Deviation)	**Preliminary Values** Abbott Architect Microalbumin Assay (mg/L), n = 6 (VCU) (Mean ± Standard Deviation)
Level I	5 mg/L to 10 mg/L	8.28 ± 0.56	9.1 ± 0.6	8.1 ± 0.2
Level II	20 mg/L to 50 mg/L	31.11 ± 1.32	34.9 ± 1.5	34.4 ± 0.1
Level III	60 mg/L to 180 mg/L	112.77 ± 5.39	105.6 ± 5.2	100.1 ± 0.9
Level IV	200 mg/L to 600 mg/L	360.50 ± 15.56	344.2 ± 19.3	354.9 ± 3.5

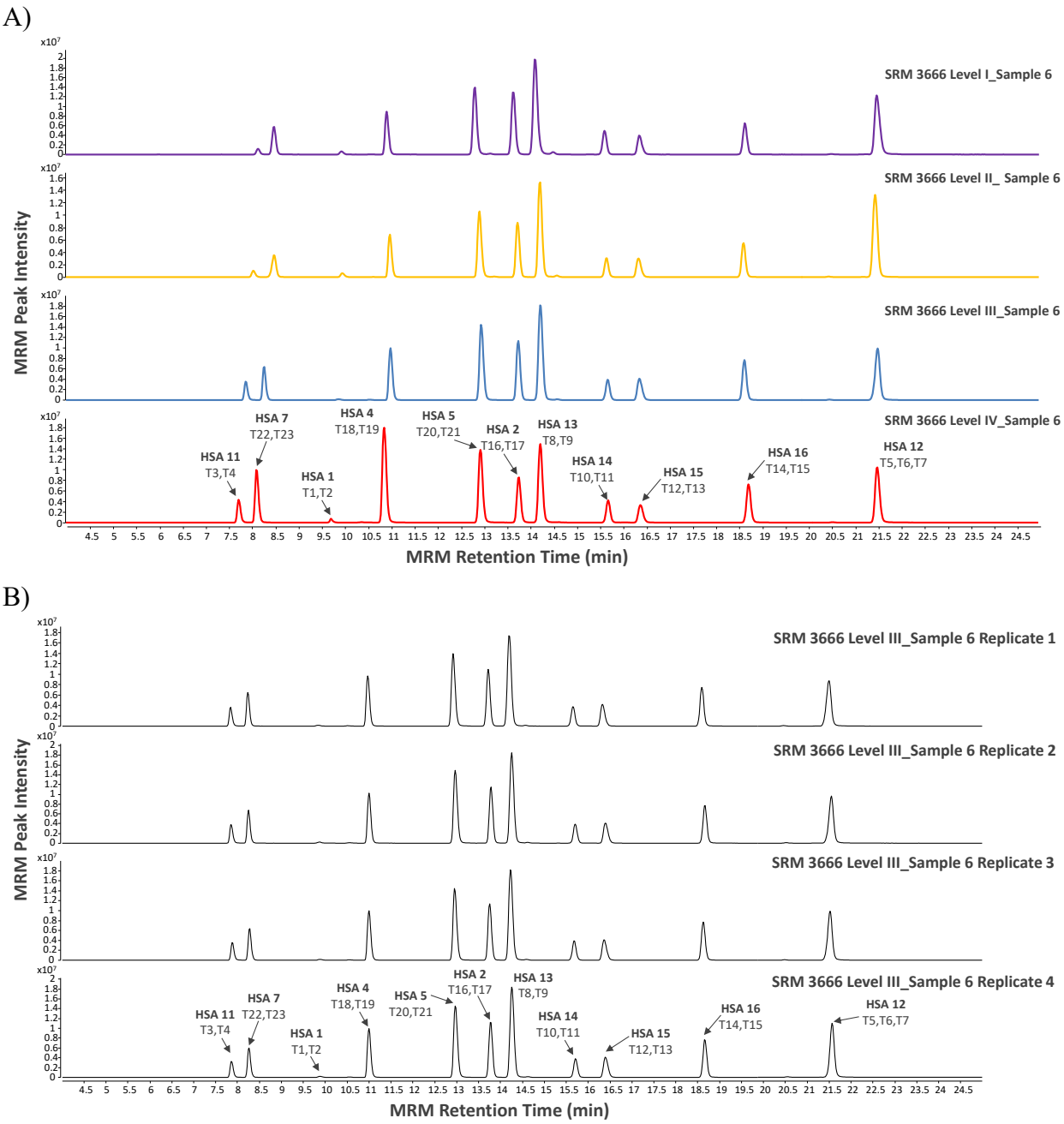

Fig. C1. Plot of MRM chromatograms containing the 11 MRM peptides for SRM 3666 selected Sample #6 (Box# 14, Vial# 1109), each level (Level I to Level IV) (A) and four technical replicates of Level III (B).

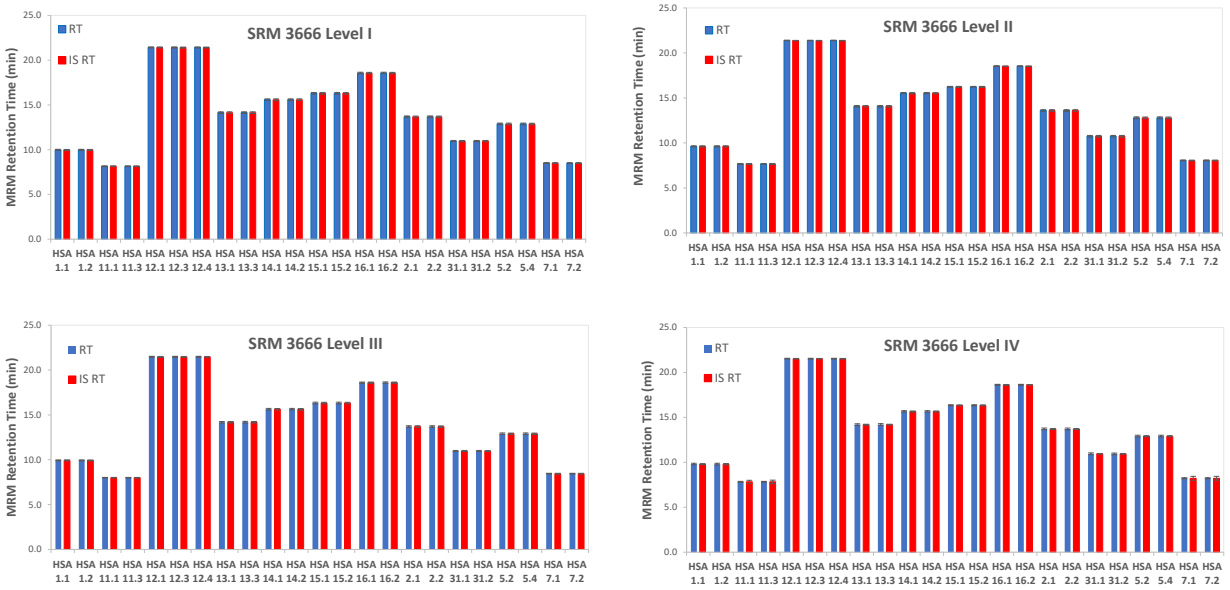

Fig. C2. MRM Retention Time (RT) profile (23 MRM Transitions) for endogenous albumin (unlabeled) and ^{15}N-Labeled recombinant HSA (IS) in SRM 3666 (Level I to Level IV). The error bars represent the standard error of the MRM RT results observed for each MRM transition. (n = 56 for each MRM transition)

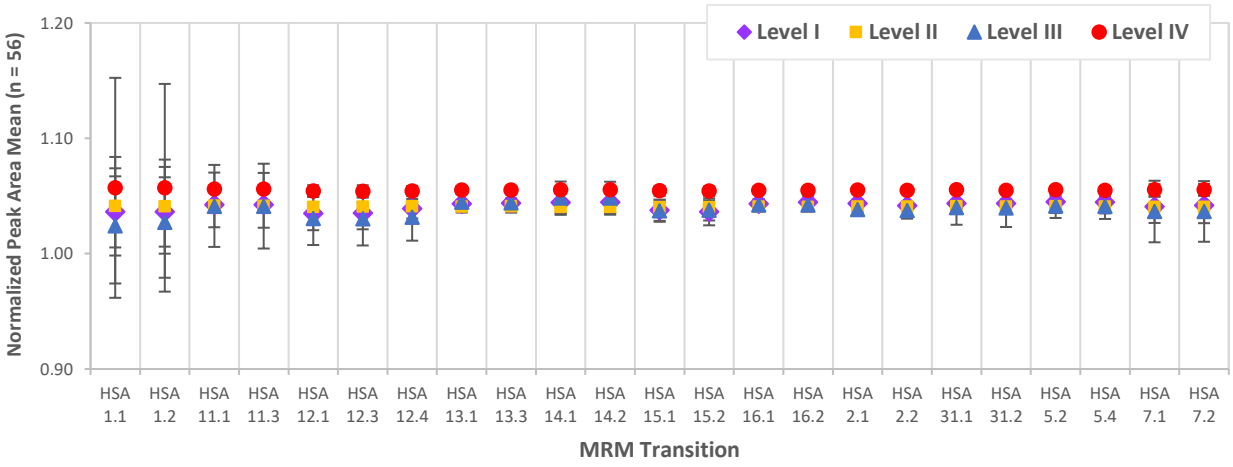

Fig. C3. Response plot of the normalized MRM peak area mean of the IS (^{15}N-labeled recombinant HSA) for each level of SRM 3666 (Level I to Level IV). The error bars represent the standard error of the MRM peak area results observed for each MRM transition (n = 56 for each MRM transition). (Level I- Purple, Level II - Yellow, Level III - Blue, and Level IV - Red)

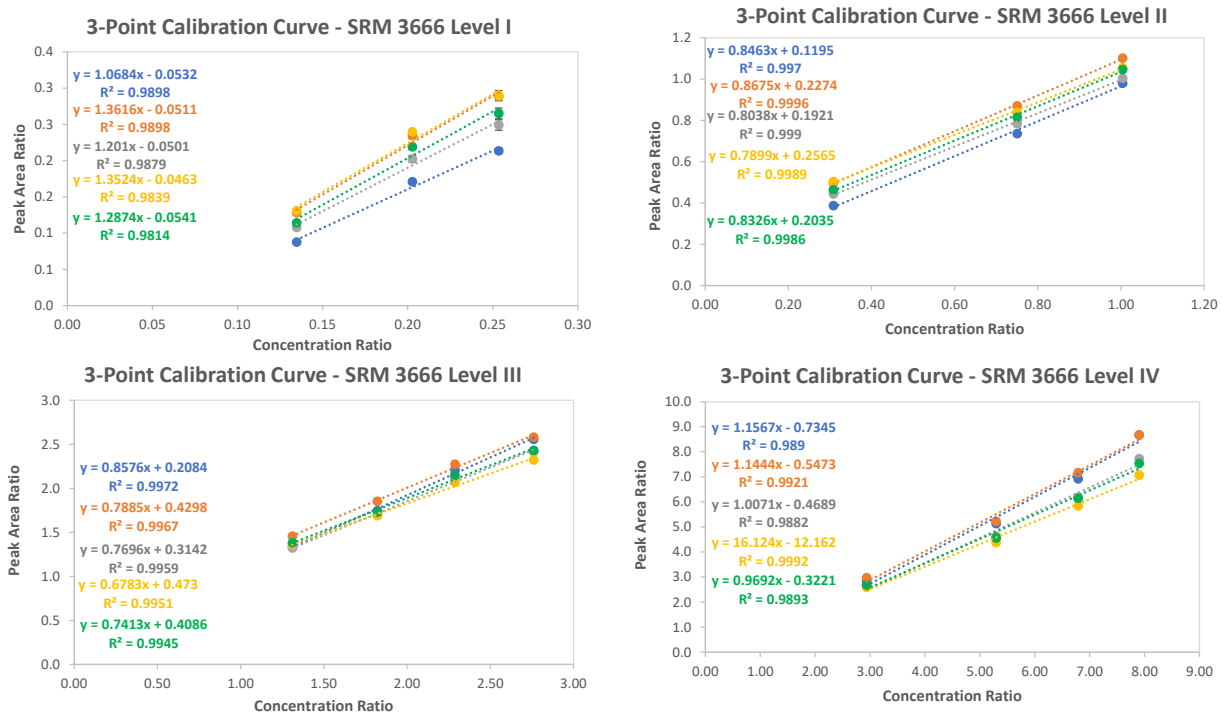

Fig. C4. Three (3)-point calibration curves for the five (5) quantitative MRM (*qt*-MRM) transitions for each level of SRM 3666 (HSA 11.1- Blue, HSA 12.4- Orange, HSA 13.3- Gray, HSA 31.1- Yellow, HSA 5.2- Green).

Appendix D. SRM 3666 – Certification of Creatinine

This section contains supplemental information to support the certification of creatinine in SRM 3666 (Level I to Level IV).

Table D1. Comparison of creatinine results for the preliminary assessment and the material certification.

SRM 3666 Level	Certified Values Mass Concentration of Creatinine (mg/dL) (NIST ID-LC-MS RMP), n = 12 (Mean ± Combined Standard Uncertainty)	Preliminary Values NIST RMP (ID-LC-MS) (mg/dL), n = 12 (Mean ± Standard Deviation)	Preliminary Values Abbott Architect Enzymatic Creatinine Assay (mg/dL), n = 6 (VCU) (Mean ± Standard Deviation)
Level I	119.69 ± 1.32	116.0 ± 0.3	116.7 ± 0.4
Level II	122.65 ± 1.36	118.0 ± 0.8	118.4 ± 0.6
Level III	127.19 ± 1.39	124.8 ± 0.6	123.0 ± 0.7
Level IV	130.90 ± 1.48	129.0 ± 0.4	127.4 ± 0.4

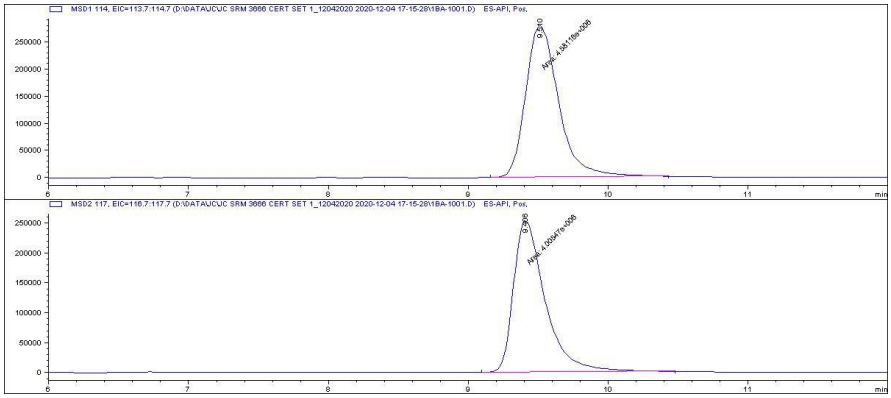

Fig. D1. Example LC-MS chromatogram of SRM 3666 Level I, box 11, vial 79, preparation 2, injection 1 (Set 1) displaying creatinine (top) and *d3*-creatinine (bottom)

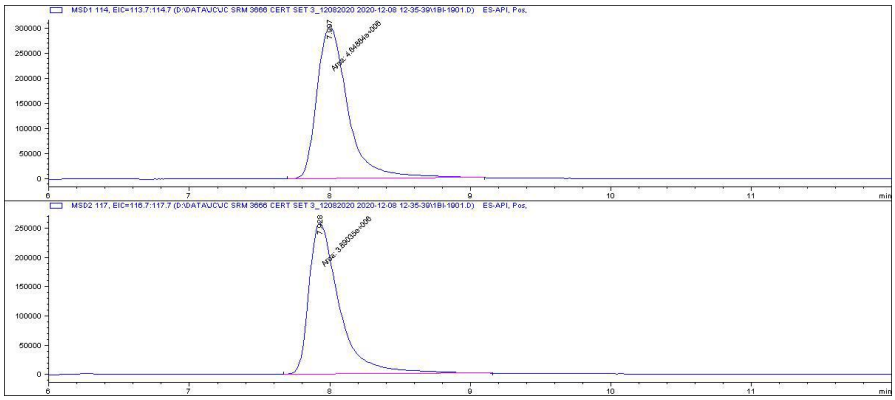

Fig. D2. Example LC-MS chromatogram of SRM 3666 Level II, box 30, vial 16, preparation 2, injection 1 (Set 3) displaying creatinine (top) and *d3*-creatinine (bottom)

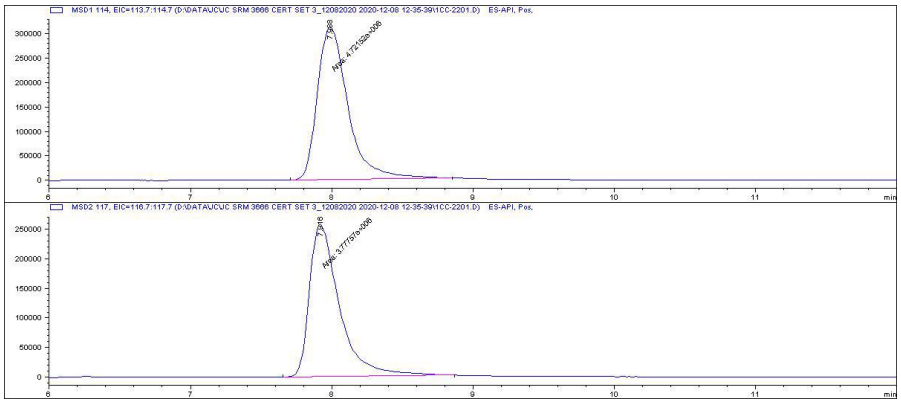

Fig. D3. Example LC-MS chromatogram of SRM 3666 Level III, box 14, vial 55, preparation 1, injection 1 (Set 3) displaying creatinine (top) and *d3*-creatinine (bottom)

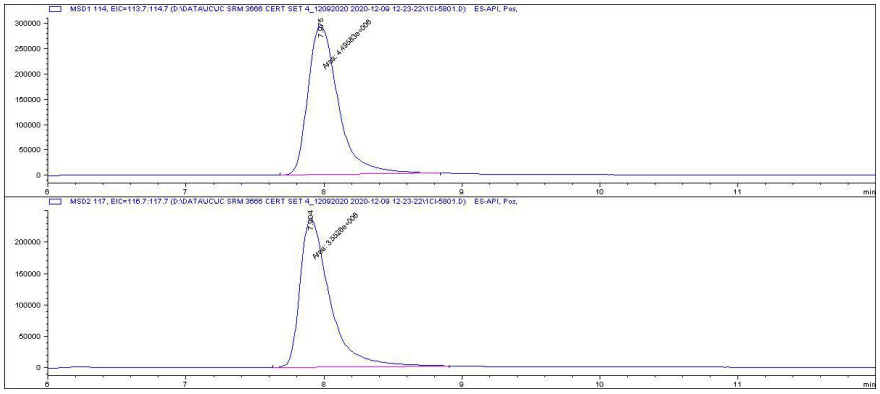

Fig. D4. Example LC-MS chromatogram of SRM 3666 Level IV, box 19, vial 65, preparation 1, injection 2 (Set 4) displaying creatinine (top) and *d3*-creatinine (bottom)

Appendix E. Homogeneity Assessment of SRM 3666

This section contains supplemental information to support the homogeneity/certification of albumin in SRM 3666 (Level I to Level IV). See also Appendices C and D for additional graphs.

E.1. Albumin Homogeneity Assessment

Appendix C includes the sampling scheme, MRM RT profile and reproducibility, and albumin calibration system. The sample size taken for the homogeneity assessment for albumin was 130 µL.

Table E1. Urine albumin measurements (PAR, mass fraction, and mass concentration values) for homogeneity assessment/certification analysis of SRM 3666 (Level I to Level IV).

						Peak Area Ratio (PAR) for qt-MRM					Mass Fraction (mg/g) Values for qt-MRM					Mass Concentration (mg/L) Values for qt-MRM				
SRM 3666 Level	Sample Label	Box #	Box Vial #	Lot Vial #	Technical Replicate	HSA 11.1	HSA 12.4	HSA 13.3	HSA 31.1	HSA 5.2	HSA 11.1	HSA 12.4	HSA 13.3	HSA 31.1	HSA 5.2	HSA 11.1	HSA 12.4	HSA 13.3	HSA 31.1	HSA 5.2
Level I	1.1	5	10	334	1	0.13	0.17	0.16	0.15	0.16	0.01	0.01	0.01	0.01	0.01	8.78	8.35	8.89	7.48	8.41
Level I	1.1	5	10	334	2	0.12	0.17	0.17	0.15	0.16	0.01	0.01	0.01	0.01	0.01	8.14	8.19	9.10	7.49	8.56
Level I	1.1	5	10	334	3	0.12	0.16	0.16	0.15	0.16	0.01	0.01	0.01	0.01	0.01	8.17	7.97	9.05	7.38	8.58
Level I	1.1	5	10	334	4	0.13	0.17	0.16	0.16	0.16	0.01	0.01	0.01	0.01	0.01	8.49	8.12	9.05	7.56	8.41
Level I	1.2	5	10	334	1	0.13	0.17	0.16	0.16	0.17	0.01	0.01	0.01	0.01	0.01	8.83	8.21	8.92	7.71	8.67
Level I	1.2	5	10	334	2	0.13	0.17	0.17	0.16	0.16	0.01	0.01	0.01	0.01	0.01	8.54	8.23	9.17	7.68	8.62
Level I	1.2	5	10	334	3	0.13	0.16	0.17	0.15	0.17	0.01	0.01	0.01	0.01	0.01	8.53	8.00	9.20	7.56	8.68
Level I	1.2	5	10	334	4	0.12	0.16	0.16	0.15	0.16	0.01	0.01	0.01	0.01	0.01	8.49	7.94	9.06	7.46	8.64
Level I	2	5	22	346	1	0.13	0.17	0.16	0.16	0.16	0.01	0.01	0.01	0.01	0.01	8.90	8.33	9.00	7.73	8.55
Level I	2	5	22	346	2	0.12	0.17	0.17	0.15	0.16	0.01	0.01	0.01	0.01	0.01	8.58	8.31	9.32	7.60	8.64
Level I	2	5	22	346	3	0.12	0.16	0.16	0.15	0.16	0.01	0.01	0.01	0.01	0.01	8.50	8.14	9.12	7.53	8.71
Level I	2	5	22	346	4	0.12	0.17	0.17	0.15	0.17	0.01	0.01	0.01	0.01	0.01	8.52	8.20	9.30	7.56	8.78
Level I	3	11	53	863	1	0.13	0.17	0.16	0.15	0.16	0.01	0.01	0.01	0.01	0.01	8.63	8.24	9.10	7.60	8.44
Level I	3	11	53	863	2	0.12	0.17	0.17	0.15	0.16	0.01	0.01	0.01	0.01	0.01	8.42	8.23	9.29	7.64	8.79
Level I	3	11	53	863	3	0.13	0.16	0.17	0.15	0.16	0.01	0.01	0.01	0.01	0.01	8.71	8.19	9.30	7.55	8.72
Level I	3	11	53	863	4	0.12	0.17	0.17	0.15	0.16	0.01	0.01	0.01	0.01	0.01	8.58	8.35	9.27	7.68	8.70
Level I	4	11	80	890	1	0.13	0.17	0.16	0.15	0.16	0.01	0.01	0.01	0.01	0.01	8.83	8.05	8.85	7.44	8.44
Level I	4	11	80	890	2	0.13	0.17	0.17	0.16	0.16	0.01	0.01	0.01	0.01	0.01	8.63	8.10	9.12	7.57	8.55
Level I	4	11	80	890	3	0.13	0.17	0.17	0.16	0.16	0.01	0.01	0.01	0.01	0.01	8.39	8.03	9.10	7.54	8.52
Level I	4	11	80	890	4	0.13	0.16	0.16	0.15	0.17	0.01	0.01	0.01	0.01	0.01	8.42	7.89	8.96	7.43	8.56
Level I	5	14	6	1059	1	0.13	0.17	0.16	0.16	0.16	0.01	0.01	0.01	0.01	0.01	8.83	8.23	9.07	7.80	8.67
Level I	5	14	6	1059	2	0.12	0.16	0.16	0.16	0.16	0.01	0.01	0.01	0.01	0.01	8.42	8.13	9.24	7.74	8.70
Level I	5	14	6	1059	3	0.12	0.16	0.16	0.15	0.16	0.01	0.01	0.01	0.01	0.01	8.42	8.02	9.17	7.54	8.73
Level I	5	14	6	1059	4	0.13	0.16	0.16	0.15	0.16	0.01	0.01	0.01	0.01	0.01	8.71	8.19	9.16	7.44	8.46
Level I	6	14	56	1109	1	0.13	0.17	0.16	0.16	0.16	0.01	0.01	0.01	0.01	0.01	8.49	8.16	8.83	7.47	8.32
Level I	6	14	56	1109	2	0.12	0.17	0.17	0.16	0.16	0.01	0.01	0.01	0.01	0.01	8.23	8.06	9.04	7.50	8.44
Level I	6	14	56	1109	3	0.12	0.16	0.16	0.15	0.16	0.01	0.01	0.01	0.01	0.01	8.15	7.80	8.89	7.40	8.36
Level I	6	14	56	1109	4	0.12	0.17	0.16	0.15	0.16	0.01	0.01	0.01	0.01	0.01	8.18	7.96	8.97	7.37	8.40
Level I	7	19	21	1479	1	0.13	0.17	0.16	0.16	0.16	0.01	0.01	0.01	0.01	0.01	8.24	7.86	8.55	7.32	8.14
Level I	7	19	21	1479	2	0.13	0.17	0.17	0.16	0.17	0.01	0.01	0.01	0.01	0.01	8.08	7.67	8.77	7.30	8.25
Level I	7	19	21	1479	3	0.13	0.17	0.17	0.16	0.17	0.01	0.01	0.01	0.01	0.01	8.25	7.85	8.76	7.26	8.30
Level I	7	19	21	1479	4	0.13	0.17	0.17	0.16	0.17	0.01	0.01	0.01	0.01	0.01	8.01	7.65	8.79	7.25	8.24
Level I	8	19	64	1522	1	0.13	0.17	0.16	0.16	0.16	0.01	0.01	0.01	0.01	0.01	8.63	8.22	9.08	7.64	8.50
Level I	8	19	64	1522	2	0.13	0.16	0.16	0.16	0.16	0.01	0.01	0.01	0.01	0.01	8.53	8.08	9.09	7.66	8.61
Level I	8	19	64	1522	3	0.13	0.17	0.17	0.15	0.16	0.01	0.01	0.01	0.01	0.01	8.52	8.12	9.19	7.56	8.56
Level I	8	19	64	1522	4	0.13	0.17	0.17	0.15	0.16	0.01	0.01	0.01	0.01	0.01	8.54	8.10	9.23	7.51	8.66
Level I	9	25	31	1975	1	0.13	0.17	0.16	0.15	0.16	0.01	0.01	0.01	0.01	0.01	8.27	8.14	8.54	7.25	8.15
Level I	9	25	31	1975	2	0.13	0.17	0.16	0.15	0.16	0.01	0.01	0.01	0.01	0.01	8.30	7.87	8.79	7.30	8.14
Level I	9	25	31	1975	3	0.13	0.16	0.16	0.15	0.16	0.01	0.01	0.01	0.01	0.01	8.30	7.75	8.80	7.27	8.24
Level I	9	25	31	1975	4	0.12	0.16	0.16	0.15	0.16	0.01	0.01	0.01	0.01	0.01	7.99	7.70	8.64	7.20	8.22
Level I	10.1	25	74	2018	1	0.13	0.17	0.16	0.16	0.16	0.01	0.01	0.01	0.01	0.01	8.56	8.30	8.84	7.58	8.39
Level I	10.1	25	74	2018	2	0.12	0.17	0.17	0.15	0.16	0.01	0.01	0.01	0.01	0.01	8.32	8.06	9.02	7.40	8.44
Level I	10.1	25	74	2018	3	0.12	0.17	0.17	0.16	0.16	0.01	0.01	0.01	0.01	0.01	8.33	8.08	9.10	7.51	8.43
Level I	10.1	25	74	2018	4	0.13	0.16	0.16	0.15	0.16	0.01	0.01	0.01	0.01	0.01	8.41	7.93	8.96	7.40	8.55
Level I	10.2	25	74	2018	1	0.13	0.18	0.16	0.15	0.16	0.01	0.01	0.01	0.01	0.01	8.54	8.46	8.83	7.50	8.30
Level I	10.2	25	74	2018	2	0.12	0.17	0.17	0.15	0.16	0.01	0.01	0.01	0.01	0.01	8.02	8.18	9.21	7.53	8.49
Level I	10.2	25	74	2018	3	0.12	0.17	0.17	0.16	0.16	0.01	0.01	0.01	0.01	0.01	8.13	8.17	9.11	7.60	8.62
Level I	10.2	25	74	2018	4	0.12	0.16	0.16	0.15	0.16	0.01	0.01	0.01	0.01	0.01	8.41	8.02	9.07	7.47	8.51
Level I	11	30	17	2366	1	0.12	0.17	0.16	0.16	0.16	0.01	0.01	0.01	0.01	0.01	8.39	8.28	8.78	7.57	8.35
Level I	11	30	17	2366	2	0.13	0.17	0.16	0.15	0.16	0.01	0.01	0.01	0.01	0.01	8.50	8.15	9.11	7.34	8.54
Level I	11	30	17	2366	3	0.12	0.17	0.16	0.15	0.16	0.01	0.01	0.01	0.01	0.01	8.29	8.09	9.07	7.47	8.62
Level I	11	30	17	2366	4	0.12	0.17	0.16	0.15	0.16	0.01	0.01	0.01	0.01	0.01	8.20	8.13	9.02	7.46	8.61
Level I	12	30	46	2395	1	0.13	0.17	0.16	0.15	0.16	0.01	0.01	0.01	0.01	0.01	8.27	8.01	8.47	7.19	8.15
Level I	12	30	46	2395	2	0.13	0.17	0.16	0.16	0.17	0.01	0.01	0.01	0.01	0.01	8.11	7.88	8.62	7.30	8.35
Level I	12	30	46	2395	3	0.12	0.17	0.17	0.15	0.16	0.01	0.01	0.01	0.01	0.01	7.97	7.75	8.71	7.12	8.21
Level I	12	30	46	2395	4	0.12	0.16	0.17	0.16	0.16	0.01	0.01	0.01	0.01	0.01	7.92	7.66	8.67	7.26	8.20
Level II	1	5	10	334	1	0.65	0.77	0.71	0.71	0.71	0.03	0.03	0.03	0.03	0.03	32.52	32.47	33.52	30.17	32.07
Level II	1	5	10	334	2	0.61	0.76	0.70	0.70	0.72	0.03	0.03	0.03	0.03	0.03	30.41	31.97	33.05	29.43	32.47
Level II	1	5	10	334	3	0.60	0.76	0.70	0.70	0.71	0.03	0.03	0.03	0.03	0.03	29.85	32.21	33.02	29.65	31.92
Level II	1	5	10	334	4	0.62	0.76	0.70	0.70	0.71	0.03	0.03	0.03	0.03	0.03	31.09	32.14	32.92	29.16	31.73
Level II	2	5	22	346	1	0.61	0.76	0.71	0.71	0.72	0.03	0.03	0.03	0.03	0.03	30.99	32.33	34.28	30.62	33.03
Level II	2	5	22	346	2	0.61	0.77	0.71	0.71	0.73	0.03	0.03	0.03	0.03	0.03	30.46	33.32	34.04	30.60	33.29
Level II	2	5	22	346	3	0.62	0.77	0.70	0.70	0.72	0.03	0.03	0.03	0.03	0.03	31.28	33.32	33.80	29.92	32.87
Level II	2	5	22	346	4	0.63	0.77	0.71	0.71	0.73	0.03	0.03	0.03	0.03	0.03	32.08	33.44	34.01	30.66	33.49
Level II	3.1	11	53	863	1	0.62	0.77	0.71	0.71	0.71	0.03	0.03	0.03	0.03	0.03	30.65	32.28	33.39	29.97	31.81
Level II	3.1	11	53	863	2	0.60	0.77	0.71	0.71	0.71	0.03	0.03	0.03	0.03	0.03	29.70	32.66	33.78	29.94	31.84
Level II	3.1	11	53	863	3	0.63	0.77	0.71	0.70	0.72	0.03	0.03	0.03	0.03	0.03	31.18	32.48	33.56	29.50	31.97
Level II	3.1	11	53	863	4	0.61	0.78	0.71	0.71	0.71	0.03	0.03	0.03	0.03	0.03	30.11	32.87	33.41	29.78	31.87
Level II	3.2	11	53	863	1	0.63	0.76	0.71	0.71	0.72	0.03	0.03	0.03	0.03	0.03	31.58	31.83	33.41	29.68	32.26
Level II	3.2	11	53	863	2	0.61	0.76	0.71	0.70	0.73	0.03	0.03	0.03	0.03	0.03	30.19	32.18	33.45	29.35	32.71
Level II	3.2	11	53	863	3	0.61	0.77	0.70	0.70	0.71	0.03	0.03	0.03	0.03	0.03	29.91	32.45	33.14	29.45	31.81
Level II	3.2	11	53	863	4	0.60	0.77	0.71	0.70	0.71	0.03	0.03	0.03	0.03	0.03	29.56	32.38	33.46	28.98	31.73
Level II	4	11	80	890	1	0.62	0.76	0.70	0.71	0.71	0.03	0.03	0.03	0.03	0.03	31.87	32.64	33.61	30.51	32.52
Level II	4	11	80	890	2	0.61	0.75	0.70	0.70	0.72	0.03	0.03	0.03	0.03	0.03	30.94	32.46	33.60	30.05	32.98
Level II	4	11	80	890	3	0.61	0.76	0.70	0.70	0.71	0.03	0.03	0.03	0.03	0.03	31.17	32.91	33.79	29.95	32.50
Level II	4	11	80	890	4	0.61	0.77	0.69	0.71	0.71	0.03	0.03	0.03	0.03	0.03	31.18	33.17	33.19	30.59	32.24
Level II	5	14	6	1059	1	0.59	0.74	0.69	0.69	0.69	0.03	0.03	0.03	0.03	0.03	29.06	30.82	31.99	28.43	30.21

Level II	5	14	6	1059	2	0.57	0.75	0.68	0.68	0.70	0.03	0.03	0.03	0.03	0.03	27.91	31.05	31.41	28.17	30.86
Level II	5	14	6	1059	3	0.58	0.75	0.68	0.68	0.70	0.03	0.03	0.03	0.03	0.03	28.41	31.19	31.71	27.88	30.75
Level II	5	14	6	1059	4	0.60	0.75	0.69	0.68	0.69	0.03	0.03	0.03	0.03	0.03	29.56	31.42	32.22	27.94	30.14
Level II	6.1	14	56	1109	1	0.62	0.76	0.69	0.70	0.69	0.03	0.03	0.03	0.03	0.03	31.07	32.03	32.68	29.16	30.91
Level II	6.1	14	56	1109	2	0.60	0.76	0.69	0.70	0.71	0.03	0.03	0.03	0.03	0.03	29.71	31.89	32.59	29.29	31.72
Level II	6.1	14	56	1109	3	0.60	0.75	0.68	0.69	0.71	0.03	0.03	0.03	0.03	0.03	29.75	31.43	32.02	28.56	31.59
Level II	6.1	14	56	1109	4	0.60	0.76	0.69	0.69	0.70	0.03	0.03	0.03	0.03	0.03	29.61	32.14	32.60	28.82	31.08
Level II	6.2	14	56	1109	1	0.59	0.75	0.70	0.69	0.72	0.03	0.03	0.03	0.03	0.03	29.16	31.48	33.12	28.93	32.28
Level II	6.2	14	56	1109	2	0.60	0.77	0.70	0.70	0.71	0.03	0.03	0.03	0.03	0.03	29.78	32.31	33.19	29.36	31.76
Level II	6.2	14	56	1109	3	0.61	0.76	0.70	0.70	0.71	0.03	0.03	0.03	0.03	0.03	29.91	31.78	32.74	29.03	31.72
Level II	6.2	14	56	1109	4	0.60	0.76	0.70	0.70	0.71	0.03	0.03	0.03	0.03	0.03	29.87	32.16	32.69	29.06	31.94
Level II	7	19	21	1479	1	0.58	0.74	0.69	0.69	0.69	0.03	0.03	0.03	0.03	0.03	28.37	30.64	32.49	28.31	30.49
Level II	7	19	21	1479	2	0.59	0.74	0.68	0.69	0.70	0.03	0.03	0.03	0.03	0.03	29.03	30.74	31.50	28.69	30.84
Level II	7	19	21	1479	3	0.61	0.75	0.68	0.68	0.69	0.03	0.03	0.03	0.03	0.03	29.86	31.22	31.69	28.11	30.68
Level II	7	19	21	1479	4	0.59	0.75	0.68	0.69	0.69	0.03	0.03	0.03	0.03	0.03	29.17	31.37	31.83	28.33	30.58
Level II	8	19	64	1522	1	0.62	0.76	0.71	0.71	0.71	0.03	0.03	0.03	0.03	0.03	30.84	32.16	33.58	29.98	31.86
Level II	8	19	64	1522	2	0.63	0.76	0.70	0.71	0.71	0.03	0.03	0.03	0.03	0.03	31.34	32.04	33.36	29.88	31.77
Level II	8	19	64	1522	3	0.60	0.76	0.70	0.70	0.71	0.03	0.03	0.03	0.03	0.03	30.01	32.27	33.32	29.60	31.87
Level II	8	19	64	1522	4	0.59	0.76	0.70	0.69	0.71	0.03	0.03	0.03	0.03	0.03	29.19	32.43	32.93	28.93	31.80
Level II	9	25	31	1975	1	0.62	0.74	0.69	0.70	0.71	0.03	0.03	0.03	0.03	0.03	30.85	30.94	31.90	28.99	31.61
Level II	9	25	31	1975	2	0.63	0.75	0.69	0.69	0.70	0.03	0.03	0.03	0.03	0.03	31.58	31.22	32.07	28.73	31.20
Level II	9	25	31	1975	3	0.62	0.74	0.69	0.69	0.70	0.03	0.03	0.03	0.03	0.03	30.59	30.93	32.28	28.42	31.11
Level II	9	25	31	1975	4	0.61	0.75	0.69	0.69	0.70	0.03	0.03	0.03	0.03	0.03	30.02	31.34	32.10	28.31	30.80
Level II	10	25	74	2018	1	0.61	0.74	0.68	0.70	0.70	0.03	0.03	0.03	0.03	0.03	29.55	30.30	31.13	28.60	30.31
Level II	10	25	74	2018	2	0.60	0.74	0.69	0.70	0.69	0.03	0.03	0.03	0.03	0.03	29.14	30.25	31.46	28.41	29.99
Level II	10	25	74	2018	3	0.60	0.74	0.68	0.69	0.70	0.03	0.03	0.03	0.03	0.03	29.17	30.41	31.01	27.97	30.59
Level II	10	25	74	2018	4	0.60	0.75	0.68	0.69	0.70	0.03	0.03	0.03	0.03	0.03	28.79	30.91	31.25	27.83	30.48
Level II	11	30	17	2366	1	0.61	0.74	0.69	0.69	0.71	0.03	0.03	0.03	0.03	0.03	30.88	31.56	32.70	29.35	32.14
Level II	11	30	17	2366	2	0.62	0.75	0.69	0.70	0.70	0.03	0.03	0.03	0.03	0.03	31.55	31.71	32.84	29.49	31.39
Level II	11	30	17	2366	3	0.60	0.74	0.70	0.69	0.70	0.03	0.03	0.03	0.03	0.03	30.39	31.62	33.20	29.05	31.45
Level II	11	30	17	2366	4	0.60	0.76	0.69	0.68	0.71	0.03	0.03	0.03	0.03	0.03	30.15	32.36	32.89	28.61	32.00
Level II	12	30	46	2395	1	0.63	0.74	0.69	0.69	0.71	0.03	0.03	0.03	0.03	0.03	31.18	30.81	32.35	28.70	31.36
Level II	12	30	46	2395	2	0.61	0.75	0.69	0.69	0.70	0.03	0.03	0.03	0.03	0.03	29.82	31.01	31.88	28.62	30.92
Level II	12	30	46	2395	3	0.62	0.76	0.69	0.69	0.70	0.03	0.03	0.03	0.03	0.03	30.79	31.54	31.92	28.60	30.91
Level II	12	30	46	2395	4	0.62	0.76	0.69	0.69	0.70	0.03	0.03	0.03	0.03	0.03	30.55	31.59	32.04	28.72	30.91
Level III	1	5	10	334	1	2.10	2.26	1.93	1.98	2.01	0.11	0.12	0.11	0.11	0.11	114.60	121.02	109.11	115.67	112.35
Level III	1	5	10	334	2	1.97	2.33	1.89	2.02	1.97	0.11	0.12	0.10	0.12	0.11	106.98	125.52	106.75	119.15	109.76
Level III	1	5	10	334	3	2.00	2.34	1.91	2.03	1.97	0.11	0.12	0.11	0.12	0.11	108.78	125.92	107.86	119.25	109.45
Level III	1	5	10	334	4	1.99	2.33	1.94	2.03	1.96	0.11	0.12	0.11	0.12	0.11	108.29	125.41	109.73	119.43	109.33
Level III	2	5	22	346	1	1.86	2.20	1.85	1.93	1.91	0.09	0.11	0.10	0.10	0.10	95.63	111.65	99.15	106.49	100.29
Level III	2	5	22	346	2	1.86	2.23	1.85	1.93	1.92	0.09	0.11	0.10	0.10	0.10	95.34	112.99	99.24	106.79	100.87
Level III	2	5	22	346	3	1.92	2.22	1.87	1.93	1.90	0.10	0.11	0.10	0.10	0.10	99.17	112.45	100.00	106.42	99.56
Level III	2	5	22	346	4	1.91	2.21	1.85	1.93	1.90	0.10	0.11	0.10	0.10	0.10	98.73	112.07	99.10	106.53	99.58
Level III	3	11	53	863	1	2.00	2.23	1.93	2.00	2.02	0.11	0.12	0.11	0.12	0.11	109.56	119.99	110.23	118.38	114.11
Level III	3	11	53	863	2	1.99	2.32	1.91	2.03	1.98	0.11	0.12	0.11	0.12	0.11	109.25	125.88	109.11	120.83	111.55
Level III	3	11	53	863	3	1.98	2.32	1.92	2.03	1.98	0.11	0.12	0.11	0.12	0.11	108.38	126.05	109.49	120.80	111.38
Level III	3	11	53	863	4	2.00	2.36	1.93	2.05	2.00	0.11	0.13	0.11	0.12	0.11	109.61	128.61	110.11	121.70	112.61
Level III	4.1	11	80	890	1	1.95	2.30	1.90	2.01	1.95	0.11	0.12	0.11	0.12	0.11	108.08	126.27	109.25	120.15	110.48
Level III	4.1	11	80	890	2	1.96	2.29	1.91	2.00	1.96	0.11	0.12	0.11	0.12	0.11	108.17	125.51	110.15	119.29	110.83
Level III	4.1	11	80	890	3	2.01	2.29	1.88	2.00	1.94	0.11	0.12	0.11	0.12	0.11	111.30	125.33	108.28	119.83	109.77
Level III	4.1	11	80	890	4	1.99	2.29	1.89	1.99	1.93	0.11	0.12	0.11	0.12	0.11	110.11	124.97	108.58	118.48	109.24
Level III	4.2	11	80	890	1	2.01	2.30	1.94	1.96	2.01	0.11	0.13	0.11	0.12	0.12	115.04	129.33	115.07	120.10	117.72
Level III	4.2	11	80	890	2	2.00	2.29	1.92	1.97	1.99	0.11	0.13	0.11	0.12	0.11	113.94	129.14	114.32	120.75	116.84
Level III	4.2	11	80	890	3	1.98	2.08	1.92	1.85	2.01	0.11	0.11	0.11	0.11	0.12	112.83	114.61	114.14	111.06	118.23
Level III	4.2	11	80	890	4	1.95	2.32	1.91	2.02	1.97	0.11	0.13	0.11	0.12	0.11	110.78	130.70	112.97	124.95	115.17
Level III	5	14	6	1059	1	2.04	2.28	1.90	1.88	1.97	0.11	0.12	0.10	0.11	0.11	110.49	121.12	106.45	107.26	109.18
Level III	5	14	6	1059	2	1.94	2.27	1.89	1.98	1.94	0.10	0.12	0.10	0.11	0.10	104.44	121.06	106.13	114.63	106.65
Level III	5	14	6	1059	3	2.01	2.26	1.90	2.00	1.92	0.11	0.12	0.10	0.11	0.10	108.91	119.99	106.47	116.20	105.38
Level III	5	14	6	1059	4	1.94	2.28	1.90	2.00	1.96	0.10	0.12	0.10	0.11	0.11	104.69	121.74	106.36	116.35	108.40
Level III	6	14	56	1109	1	1.93	2.28	1.87	1.98	1.92	0.10	0.12	0.10	0.11	0.10	105.18	122.78	105.67	116.15	106.80
Level III	6	14	56	1109	2	1.90	2.27	1.87	1.98	1.91	0.10	0.12	0.10	0.11	0.10	103.20	121.77	105.98	116.35	105.99
Level III	6	14	56	1109	3	1.90	2.26	1.87	1.97	1.92	0.10	0.12	0.10	0.11	0.10	103.16	121.58	105.66	115.05	106.42
Level III	6	14	56	1109	4	1.96	2.28	1.86	1.98	1.92	0.11	0.12	0.10	0.11	0.10	106.88	122.69	105.33	116.23	106.41
Level III	7	19	21	1479	1	1.96	2.26	1.90	2.03	1.95	0.11	0.12	0.11	0.12	0.11	107.43	122.52	108.99	120.92	109.60
Level III	7	19	21	1479	2	1.99	2.18	1.90	1.94	1.98	0.11	0.12	0.11	0.11	0.11	109.68	117.25	108.94	113.91	112.05
Level III	7	19	21	1479	3	1.96	2.28	1.89	2.02	1.95	0.11	0.12	0.11	0.12	0.11	107.86	123.96	108.18	120.48	110.00
Level III	7	19	21	1479	4	2.00	2.33	1.89	2.01	1.94	0.11	0.13	0.11	0.12	0.11	110.18	127.27	107.88	119.47	108.67
Level III	8.1	19	64	1522	1	1.97	2.30	1.91	1.96	1.99	0.11	0.12	0.11	0.11	0.11	106.89	123.48	108.31	113.85	111.31
Level III	8.1	19	64	1522	2	1.93	2.26	1.90	2.00	1.97	0.10	0.12	0.11	0.11	0.11	104.82	120.88	107.36	117.04	109.70
Level III	8.1	19	64	1522	3	1.95	2.26	1.88	1.99	1.95	0.10	0.12	0.10	0.11	0.11	105.49	121.09	106.09	116.71	108.58
Level III	8.1	19	64	1522	4	1.92	2.24	1.89	2.00	1.95	0.10	0.12	0.10	0.12	0.11	103.99	119.52	106.40	117.45	108.14
Level III	8.2	19	64	1522	1	1.96	2.26	1.88	2.00	1.97	0.10	0.12	0.10	0.12	0.11	106.34	120.84	105.55	117.27	109.23
Level III	8.2	19	64	1522	2	1.93	2.29	1.88	2.01	1.96	0.10	0.12	0.10	0.12	0.11	104.70	122.40	105.67	117.57	109.01
Level III	8.2	19	64	1522	3	1.97	2.28	1.91	2.02	1.95	0.10	0.12	0.11	0.12	0.11	106.60	121.83	107.82	118.48	108.36
Level III	8.2	19	64	1522	4	1.95	2.28	1.89	1.99	1.95	0.10	0.12	0.10	0.11	0.11	105.54	121.89	106.26	116.68	108.34
Level III	9	25	31	1975	1	1.99	2.37	1.92	2.04	1.98	0.10	0.12	0.10	0.12	0.11	106.59	126.00	106.87	117.99	108.29
Level III	9	25	31	1975	2	1.94	2.34	1.90	2.02	1.98	0.10	0.12	0.10	0.11	0.11	103.41	123.87	105.77	116.99	108.63
Level III	9	25	31	1975	3	2.01	2.35	1.89	2.01	2.01	0.11	0.12	0.10	0.11	0.11	107.31	124.76	104.68	116.18	110.49
Level III	9	25	31	1975	4	1.91	2.27	1.90	1.99	2.01	0.10	0.12	0.10	0.11	0.11	101.62	119.43	105.29	114.62	110.78
Level III	10	25	74	2018	1	1.99	2.37	1.91	2.01	1.98	0.11	0.12	0.11	0.12	0.11	107.04	126.66	107.00	117.16	109.64
Level III	10	25	74	2018	2	1.98	2.35	1.91	2.02	1.98	0.10	0.12	0.11	0.12	0.11	106.58	125.74	107.21	117.74	109.14
Level III	10	25	74	2018	3	2.02	2.29	1.90	1.95	1.98	0.11	0.12	0.10	0.11	0.11	109.07	121.71	105.92	112.37	109.36
Level III	10	25	74	2018	4	1.92	2.37	1.90	2.03	1.99	0.10	0.12	0.10	0.12	0.11	102.89	126.76	106.14	118.25	110.18
Level III	11	30	17	2366	1	1.90	2.38	1.92	2.01	1.96	0.10	0.13	0.11	0.12	0.11	104.45	131.04	110.64	120.65	111.23
Level III	11	30	17	2366	2	1.92	2.35	1.88	2.00	1.98	0.10	0.13	0.11	0.12	0.11	105.92	129.51	107.95	119.87	112.54
Level III	11	30	17	2366	3	1.93	2.32	1.88	1.98	1.96	0.11	0.13	0.11	0.12	0.11	106.89	127.33	107.88	118.10	111.40
Level III	11	30	17	2366	4	1.93	2.33	1.91	2.00	1.95	0.10	0.13	0.11	0.12	0.11	106.58	127.96	109.77	119.65	110.24
Level III	12	30	46	2395	1	2.00	2.42	1.88	1.98	2.00	0.11	0.13	0.10	0.11	0.11	108.81	131.61	106.39	116.21	111.93
Level III	12	30	46	2395	2	1.98	2.35	1.88	2.04	1.97	0.11	0.12	0.10	0.12	0.11	107.56	127.14	105.96	120.42	110.15
Level III	12	30	46	2395	3	1.99	2.37	1.90	2.04	1.98	0.11	0.13	0.11	0.12	0.11	108.44	128.09	107.26	120.48	110.39
Level III	12	30	46	2395	4	1.95	2.23	1.88	2.03	1.97	0.10	0.12	0.10	0.12	0.11	105.78	119.21	106.38	119.39	109.75
Level IV	1	5	10	334	1	7.16	7.38	6.41	6.33	6.49	0.34	0.36	0.34	0.36	0.34	346.86	364.04	345.20	360.88	345.88
Level IV	1	5	10	334	2	7.13	7.40	6.37	6.26	6.43	0.34	0.36	0.34	0.35	0.34	345.40	365.03	343.10	356.51	342.40
Level IV	1	5	10	334	3	7.04	7.21	6.42	6.26	6.45	0.34	0.35	0.34	0.35	0.34	340.83	355.69	346.08	356.48	343.30
Level IV	1	5	10	334	4	7.17	7.28	6.38	6.24	6.54	0.34	0.35	0.34	0.35	0.34	347.02	359.18	343.47	355.42	348.82
Level IV	2.1	5	22	346	1	7.45	7.28	6.42	6.45	6.71	0.37	0.36	0.35	0.37	0.36	371.90	370.07	356.42	379.75	369.22
Level IV	2.1	5	22	346	2	7.37	7.28	6.66	6.59	6.61	0.36	0.36	0.36	0.38	0.36	368.12	370.46	370.17	388.53	363.38
Level IV	2.1	5	22	346	3	7.25	7.30	6.50	6.55	6.75	0.36	0.37	0.36	0.38	0.37	361.96	371.31	361.08	385.61	371.45
Level IV	2.1	5	22	346	4	7.53	6.92	6.49	6.51	6.58	0.37	0.35	0.35	0.38	0.36	375.87	351.24	360.31	383.53	362.06
Level IV	2.2	5	22	346	1	7.31	7.17	6.38	6.20	6.35	0.37	0.37	0.36	0.37	0.35	372.53	372.11	361.67	371.29	355.75
Level IV	2.2	5	22	346	2	7.23	7.17	6.40	6.19	6.57	0.36	0.37	0.36	0.37	0.36	368.65	371.94	362.59	371.18	368.50
Level IV	2.2	5	22	346	3	7.24	7.17	6.37	6.25	6.43	0.36	0.37	0.36	0.37	0.35	368.93	372.09	360.85	374.51	360.12
Level IV	2.2	5	22	346	4	7.16	7.08	6.34	6.21	6.43	0.36	0.36	0.35	0.37	0.36	364.89	366.95	359.19	371.84	360.68
Level IV	3	11	53	863	1	7.18	6.99	6.17	5.93	6.24	0.36	0.36	0.35	0.35	0.35	370.39	366.52	353.83	358.57	353.60
Level IV	3	11	53	863	2	7.28	6.89	6.27	6.01	6.26	0.37	0.36	0.35	0.36	0.35	375.54	360.79	359.99	364.14	354.46
Level IV	3	11	53	863	3	7.14	6.85	6.20	5.95	6.24	0.36	0.35	0.35	0.35	0.35	368.39	359.10	355.82	360.02	353.59
Level IV	3	11	53	863	4	7.32	6.84	6.13	6.01	6.27	0.37	0.35	0.35	0.36	0.35	377.57	358.20	351.55	363.73	355.30
Level IV	4	11	80	890	1	7.39	7.32	6.51	6.37	6.65	0.34	0.35	0.34	0.35	0.34	347.48	350.73	341.08	352.99	344.63
Level IV	4	11	80	890	2	7.50	7.25	6.41	6.27	6.51	0.35	0.34	0.33	0.34	0.33	352.79	347.45	335.40	347.05	336.99
Level IV	4	11	80	890	3	7.65	7.37	6.58	6.35	6.62	0.35	0.35	0.34	0.35	0.34	359.73	353.70	344.37	351.67	343.09
Level IV	4	11	80	890	4	7.44	7.19	6.44	6.37	6.63	0.34	0.34	0.33	0.35	0.34	350.20	344.37	337.00	353.02	343.76
Level IV	5	14	6	1059	1	7.33	7.13	6.43	6.14	6.41	0.37	0.36	0.36	0.36	0.35	373.51	369.47	364.18	367.80	359.08
Level IV	5	14	6	1059	2	7.55	7.22	6.47	6.21	6.61	0.38	0.37	0.36	0.37	0.37	384.39	374.23	366.66	371.76	370.71
Level IV	5	14	6	1059	3	7.20	7.24	6.46	6.23	6.55	0.36	0.37	0.36	0.37	0.36	366.91	375.78	366.39	373.11	367.50
Level IV	5	14	6	1059	4	7.43	7.11	6.49	6.15	6.47	0.37	0.36	0.36	0.36	0.36	378.57	368.63	367.73	368.50	362.55
Level IV	6	14	56	1109	1	7.43	7.52	6.62	6.41	6.57	0.36	0.37	0.36	0.36	0.35	364.52	376.84	361.96	370.57	355.18
Level IV	6	14	56	1109	2	7.62	7.52	6.70	6.49	6.71	0.37	0.37	0.36	0.37	0.36	374.21	376.68	366.08	375.80	363.03

Level IV	6	14	56	1109	3	7.57	7.57	6.50	6.44	6.77	0.37	0.37	0.35	0.37	0.36	371.59	379.53	355.29	372.56	366.42
Level IV	6	14	56	1109	4	7.51	7.50	6.60	6.41	6.71	0.36	0.37	0.35	0.37	0.36	368.41	375.52	360.49	371.03	363.35
Level IV	7	19	21	1479	1	7.64	6.95	6.48	6.00	6.45	0.37	0.34	0.35	0.34	0.34	372.00	344.00	351.00	342.85	345.54
Level IV	7	19	21	1479	2	7.97	6.88	6.68	6.06	6.51	0.38	0.34	0.36	0.34	0.34	387.85	340.42	362.05	346.51	348.68
Level IV	7	19	21	1479	3	7.70	6.83	6.41	5.98	6.30	0.37	0.33	0.34	0.34	0.33	374.86	337.45	347.33	341.34	337.25
Level IV	7	19	21	1479	4	7.75	6.86	6.60	6.02	6.44	0.37	0.33	0.35	0.34	0.34	377.46	339.19	357.46	344.15	344.97
Level IV	8	19	64	1522	1	7.56	7.34	6.73	6.46	6.63	0.36	0.35	0.35	0.36	0.35	362.75	359.14	359.53	365.66	350.37
Level IV	8	19	64	1522	2	7.58	7.40	6.58	6.44	6.68	0.36	0.36	0.35	0.36	0.35	363.82	362.05	351.46	364.37	353.58
Level IV	8	19	64	1522	3	7.54	7.52	6.58	6.44	6.76	0.36	0.36	0.35	0.36	0.35	361.60	368.28	351.19	363.99	357.99
Level IV	8	19	64	1522	4	7.68	7.74	6.68	6.50	6.82	0.36	0.37	0.35	0.36	0.36	368.39	379.28	357.14	367.74	360.98
Level IV	9	25	31	1975	1	6.92	6.97	6.17	6.16	6.32	0.35	0.36	0.35	0.37	0.35	359.36	367.71	355.82	376.08	360.32
Level IV	9	25	31	1975	2	7.08	6.51	6.17	6.08	6.36	0.36	0.34	0.35	0.37	0.36	367.59	342.43	355.87	370.67	362.97
Level IV	9	25	31	1975	3	7.11	6.88	6.15	6.10	6.36	0.36	0.36	0.35	0.37	0.36	369.30	362.79	354.92	372.19	362.62
Level IV	9	25	31	1975	4	7.13	7.06	6.29	6.04	6.26	0.36	0.37	0.36	0.36	0.35	370.24	372.55	363.19	368.02	356.80
Level IV	10	25	74	2018	1	7.21	6.94	6.30	6.15	6.36	0.35	0.34	0.34	0.35	0.34	355.62	347.86	345.82	356.86	345.00
Level IV	10	25	74	2018	2	7.11	7.12	6.17	6.15	6.31	0.35	0.35	0.33	0.35	0.34	351.10	357.79	338.49	356.99	342.05
Level IV	10	25	74	2018	3	7.25	6.89	6.22	6.18	6.41	0.35	0.34	0.34	0.35	0.34	357.84	345.50	341.56	358.51	347.95
Level IV	10	25	74	2018	4	7.23	7.11	6.44	6.16	6.41	0.35	0.35	0.35	0.35	0.34	356.79	356.99	353.38	357.08	348.04
Level IV	11	30	17	2366	1	7.95	6.94	6.78	6.24	6.58	0.38	0.33	0.36	0.35	0.34	383.54	340.17	364.24	354.42	349.85
Level IV	11	30	17	2366	2	7.79	7.22	6.82	6.29	6.57	0.37	0.35	0.36	0.35	0.34	375.54	354.88	366.20	357.34	349.19
Level IV	11	30	17	2366	3	7.98	7.29	6.79	6.30	6.60	0.38	0.35	0.36	0.35	0.35	384.75	358.52	364.85	357.92	350.81
Level IV	11	30	17	2366	4	8.11	7.12	6.80	6.28	6.69	0.38	0.34	0.36	0.35	0.35	390.96	349.79	365.41	356.83	356.03
Level IV	12.1	30	46	2395	1	8.09	7.54	7.09	6.65	6.96	0.37	0.35	0.36	0.36	0.35	371.07	352.93	362.38	360.58	352.86
Level IV	12.1	30	46	2395	2	8.23	7.42	6.99	6.62	6.93	0.37	0.34	0.35	0.35	0.35	377.52	347.26	357.45	358.86	351.35
Level IV	12.1	30	46	2395	3	7.91	7.49	7.04	6.62	6.92	0.36	0.35	0.35	0.35	0.35	363.06	350.74	360.09	358.70	350.57
Level IV	12.1	30	46	2395	4	7.91	7.78	6.90	6.66	6.94	0.36	0.36	0.35	0.36	0.35	363.11	365.10	352.55	360.81	351.75
Level IV	12.2	30	46	2395	1	7.14	7.20	6.35	6.13	6.41	0.36	0.37	0.36	0.36	0.36	366.40	376.32	362.72	369.58	361.73
Level IV	12.2	30	46	2395	2	7.29	6.96	6.30	6.24	6.35	0.37	0.36	0.35	0.37	0.35	374.24	362.93	359.44	376.75	358.18
Level IV	12.2	30	46	2395	3	7.27	7.13	6.25	6.13	6.27	0.37	0.37	0.35	0.36	0.35	373.32	372.62	356.85	369.74	353.79
Level IV	12.2	30	46	2395	4	7.24	7.15	6.32	6.20	6.26	0.37	0.37	0.36	0.37	0.35	371.67	373.35	360.99	374.50	353.19

Table E2. Average results of urine albumin measurements (means of PAR, mass fraction, and mass concentration values from Table E1 with standard deviation and %CV).

SRM 366 Level #	Mean Peak Area Ratio (PAR) (n = 280)	Stdev	%CV	Mean Mass Fraction (mg/g) (n = 280)	Stdev	%CV	Mean Mass Concentration (mg/dL) (n = 280)	Stdev	%CV
Level I	0.15	0.02	10.2	0.01	0.00	6.5	8.28	0.54	6.5
Level II	0.69	0.05	7.0	0.03	0.00	4.9	31.11	1.52	4.9
Level III	2.02	0.15	7.2	0.11	0.01	6.7	112.77	7.52	6.7
Level IV	6.78	0.51	7.5	0.36	0.01	3.1	360.56	11.20	3.1

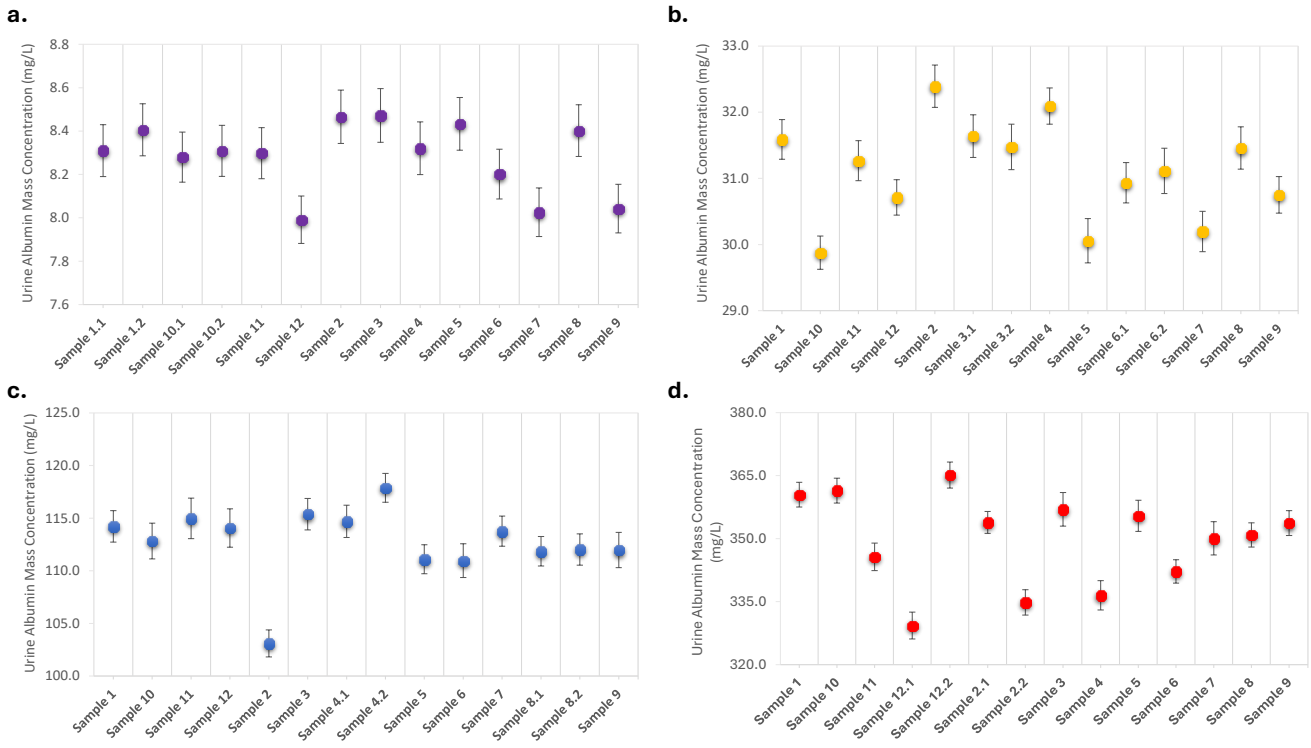

Fig. E1. Plots of sample number (vial number) versus measured endogenous albumin content value for SRM 3666 Level I (a), Level II (b), Level III (c), and Level IV (d). The error bars represent the standard error of the endogenous albumin content in SRM 3666 Level I to Level IV (n = 20) observed for each sample.

E.2. Creatinine Homogeneity Assessment

The sampling scheme for creatinine is included in Appendix C. The sample size taken for the homogeneity assessment for creatinine was 50 µL.

Table E3. Urine creatinine measurements (MRM peak area, PAR, mass fraction, and mass concentration values) for homogeneity assessment/certification analysis of SRM 3666 (Level I to Level IV).

SRM 3666 Level	Sample Name	Box #	Vial #	Preparation # (Process Rep.)	Injection # (Technical Rep.)	Creatinine at *m/z* 114	d_3-Creatinine at *m/z* 117	Peak Area Ratio (PAR)	Mass Fraction of Creatinine in Sample (µg/g)	Mass Concentration of Creatinine in Sample (mg/dL)
Level I	Sample 1	5	11	1	1	4.32E+06	3.16E+06	1.3659	1183.1	120.10
Level I	Sample 1	5	11	1	2	4.53E+06	3.30E+06	1.3729	1189.1	120.72
Level I	Sample 1	5	11	2	1	4.38E+06	3.17E+06	1.3830	1182.2	120.01
Level I	Sample 1	5	11	2	2	4.54E+06	3.26E+06	1.3911	1189.1	120.71
Level I	Sample 2	5	23	1	1	4.28E+06	3.21E+06	1.3355	1176.4	119.43
Level I	Sample 2	5	23	1	2	4.46E+06	3.31E+06	1.3499	1189.1	120.72
Level I	Sample 2	5	23	2	1	4.37E+06	3.22E+06	1.3570	1182.2	120.01
Level I	Sample 2	5	23	2	2	4.58E+06	3.36E+06	1.3619	1186.5	120.45
Level I	Sample 3	11	54	1	1	4.60E+06	4.08E+06	1.1271	1170.8	118.86
Level I	Sample 3	11	54	1	2	4.88E+06	4.31E+06	1.1321	1175.9	119.38
Level I	Sample 3	11	54	2	1	4.55E+06	4.04E+06	1.1252	1168.8	118.66
Level I	Sample 3	11	54	2	2	4.80E+06	4.25E+06	1.1294	1173.2	119.10
Level I	Sample 4	11	79	1	1	4.66E+06	4.08E+06	1.1415	1170.2	118.80
Level I	Sample 4	11	79	1	2	4.88E+06	4.28E+06	1.1398	1168.5	118.62
Level I	Sample 4	11	79	2	1	4.65E+06	4.07E+06	1.1431	1170.7	118.85
Level I	Sample 4	11	79	2	2	4.87E+06	4.27E+06	1.1391	1166.6	118.43
Level I	Sample 5	14	7	1	1	4.65E+06	4.06E+06	1.1468	1164.7	118.24
Level I	Sample 5	14	7	1	2	4.74E+06	4.16E+06	1.1390	1172.2	119.00
Level I	Sample 5	14	7	2	1	4.61E+06	3.99E+06	1.1541	1189.0	120.70
Level I	Sample 5	14	7	2	2	4.72E+06	4.16E+06	1.1343	1168.5	118.63
Level I	Sample 6	14	55	1	1	4.57E+06	4.07E+06	1.1219	1168.8	118.66
Level I	Sample 6	14	55	1	2	4.65E+06	4.13E+06	1.1267	1173.8	119.17
Level I	Sample 6	14	55	2	1	4.64E+06	4.05E+06	1.1456	1174.7	119.25
Level I	Sample 6	14	55	2	2	4.73E+06	4.15E+06	1.1394	1168.3	118.60
Level I	Sample 7	19	20	1	1	4.20E+06	3.71E+06	1.1321	1192.0	121.01
Level I	Sample 7	19	20	1	2	4.25E+06	3.75E+06	1.1336	1193.6	121.17
Level I	Sample 7	19	20	2	1	4.25E+06	3.73E+06	1.1400	1189.3	120.74
Level I	Sample 7	19	20	2	2	4.27E+06	3.76E+06	1.1348	1183.8	120.18
Level I	Sample 8	19	65	1	1	4.25E+06	3.70E+06	1.1466	1192.7	121.08
Level I	Sample 8	19	65	1	2	4.25E+06	3.73E+06	1.1397	1185.6	120.36
Level I	Sample 8	19	65	2	1	4.21E+06	3.72E+06	1.1305	1179.6	119.75
Level I	Sample 8	19	65	2	2	4.20E+06	3.70E+06	1.1329	1182.1	120.00
Level I	Sample 9	25	32	1	1	4.63E+06	4.10E+06	1.1282	1170.9	118.87
Level I	Sample 9	25	32	1	2	4.89E+06	4.31E+06	1.1339	1176.8	119.46
Level I	Sample 9	25	32	2	1	4.62E+06	4.11E+06	1.1244	1174.7	119.25
Level I	Sample 9	25	32	2	2	4.85E+06	4.30E+06	1.1265	1176.8	119.47
Level I	Sample 10	25	76	1	1	4.26E+06	3.17E+06	1.3466	1171.6	118.94
Level I	Sample 10	25	76	1	2	4.49E+06	3.29E+06	1.3659	1188.4	120.65
Level I	Sample 10	25	76	2	1	4.29E+06	3.16E+06	1.3599	1181.6	119.96
Level I	Sample 10	25	76	2	2	4.50E+06	3.28E+06	1.3729	1192.9	121.11
Level I	Sample 11	30	16	1	1	4.63E+06	4.05E+06	1.1447	1168.6	118.63
Level I	Sample 11	30	16	1	2	4.72E+06	4.13E+06	1.1437	1167.6	118.53
Level I	Sample 11	30	16	2	1	4.59E+06	4.00E+06	1.1472	1181.3	119.93
Level I	Sample 11	30	16	2	2	4.69E+06	4.11E+06	1.1398	1173.7	119.15
Level I	Sample 12	30	45	1	1	4.27E+06	3.75E+06	1.1392	1188.4	120.65
Level I	Sample 12	30	45	1	2	4.27E+06	3.72E+06	1.1494	1199.1	121.73
Level I	Sample 12	30	45	2	1	4.24E+06	3.71E+06	1.1453	1190.4	120.84
Level I	Sample 12	30	45	2	2	4.28E+06	3.78E+06	1.1328	1177.4	119.53
Level II	Sample 1	5	11	1	1	4.18E+06	3.02E+06	1.3829	1213.3	123.03
Level II	Sample 1	5	11	1	2	4.34E+06	3.10E+06	1.3981	1226.7	124.38
Level II	Sample 1	5	11	2	1	4.22E+06	2.98E+06	1.4162	1223.1	124.02
Level II	Sample 1	5	11	2	2	4.36E+06	3.09E+06	1.4123	1219.8	123.68
Level II	Sample 2	5	23	1	1	4.21E+06	3.02E+06	1.3966	1215.9	123.29
Level II	Sample 2	5	23	1	2	4.34E+06	3.09E+06	1.4049	1223.1	124.02
Level II	Sample 2	5	23	2	1	4.27E+06	3.00E+06	1.4229	1219.4	123.64
Level II	Sample 2	5	23	2	2	4.40E+06	3.07E+06	1.4333	1228.4	124.55
Level II	Sample 3	11	54	1	1	4.50E+06	4.08E+06	1.1020	1198.0	121.48
Level II	Sample 3	11	54	1	2	4.68E+06	4.24E+06	1.1036	1199.8	121.65
Level II	Sample 3	11	54	2	1	4.71E+06	4.06E+06	1.1608	1197.5	121.43
Level II	Sample 3	11	54	2	2	4.89E+06	4.21E+06	1.1636	1200.4	121.71
Level II	Sample 4	11	79	1	1	4.69E+06	4.00E+06	1.1728	1196.4	121.31
Level II	Sample 4	11	79	1	2	4.88E+06	4.15E+06	1.1738	1197.4	121.41
Level II	Sample 4	11	79	2	1	4.68E+06	4.01E+06	1.1673	1199.2	121.59
Level II	Sample 4	11	79	2	2	4.88E+06	4.19E+06	1.1641	1195.9	121.26
Level II	Sample 5	14	7	1	1	4.51E+06	3.89E+06	1.1614	1195.6	121.23
Level II	Sample 5	14	7	1	2	4.56E+06	3.84E+06	1.1856	1220.5	123.76
Level II	Sample 5	14	7	2	1	4.51E+06	3.87E+06	1.1651	1197.6	121.43
Level II	Sample 5	14	7	2	2	4.57E+06	3.87E+06	1.1821	1215.0	123.19
Level II	Sample 6	14	55	1	1	4.61E+06	3.88E+06	1.1877	1189.3	120.59
Level II	Sample 6	14	55	1	2	4.64E+06	3.86E+06	1.2013	1202.9	121.97
Level II	Sample 6	14	55	2	1	4.47E+06	3.82E+06	1.1687	1188.6	120.52
Level II	Sample 6	14	55	2	2	4.53E+06	3.84E+06	1.1788	1198.8	121.55
Level II	Sample 7	19	20	1	1	4.14E+06	3.50E+06	1.1842	1218.9	123.59
Level II	Sample 7	19	20	1	2	4.09E+06	3.43E+06	1.1946	1229.6	124.68
Level II	Sample 7	19	20	2	1	4.10E+06	3.45E+06	1.1884	1221.0	123.81

Level II	Sample 7	19	20	2	2	4.09E+06	3.44E+06	1.1872	1219.8	123.69
Level II	Sample 8	19	65	1	1	4.20E+06	3.52E+06	1.1933	1229.1	124.63
Level II	Sample 8	19	65	1	2	4.12E+06	3.45E+06	1.1943	1230.1	124.73
Level II	Sample 8	19	65	2	1	4.11E+06	3.45E+06	1.1917	1220.8	123.78
Level II	Sample 8	19	65	2	2	4.12E+06	3.47E+06	1.1875	1216.4	123.34
Level II	Sample 9	25	32	1	1	4.66E+06	4.00E+06	1.1650	1195.4	121.21
Level II	Sample 9	25	32	1	2	4.86E+06	4.16E+06	1.1686	1199.1	121.59
Level II	Sample 9	25	32	2	1	4.76E+06	4.06E+06	1.1731	1193.4	121.00
Level II	Sample 9	25	32	2	2	4.87E+06	4.16E+06	1.1701	1190.3	120.70
Level II	Sample 10	25	76	1	1	4.12E+06	2.98E+06	1.3819	1216.4	123.34
Level II	Sample 10	25	76	1	2	4.33E+06	3.12E+06	1.3882	1222.0	123.91
Level II	Sample 10	25	76	2	1	4.18E+06	2.98E+06	1.4025	1213.8	123.08
Level II	Sample 10	25	76	2	2	4.35E+06	3.08E+06	1.4126	1222.6	123.97
Level II	Sample 11	30	16	1	1	4.66E+06	4.08E+06	1.1426	1185.1	120.16
Level II	Sample 11	30	16	1	2	4.52E+06	3.88E+06	1.1649	1208.2	122.51
Level II	Sample 11	30	16	2	1	4.50E+06	3.89E+06	1.1580	1195.6	121.23
Level II	Sample 11	30	16	2	2	4.48E+06	3.87E+06	1.1572	1194.8	121.15
Level II	Sample 12	30	45	1	1	4.01E+06	3.48E+06	1.1525	1211.3	122.83
Level II	Sample 12	30	45	1	2	4.06E+06	3.49E+06	1.1615	1220.7	123.78
Level II	Sample 12	30	45	2	1	4.12E+06	3.50E+06	1.1768	1218.1	123.51
Level II	Sample 12	30	45	2	2	4.19E+06	3.55E+06	1.1804	1221.9	123.89
Level III	Sample 1	5	11	1	1	4.35E+06	2.98E+06	1.4585	1258.4	128.09
Level III	Sample 1	5	11	1	2	4.45E+06	3.05E+06	1.4619	1261.3	128.38
Level III	Sample 1	5	11	2	1	4.38E+06	3.01E+06	1.4548	1259.9	128.24
Level III	Sample 1	5	11	2	2	4.47E+06	3.06E+06	1.4636	1267.5	129.01
Level III	Sample 2	5	23	1	1	4.39E+06	3.02E+06	1.4558	1258.9	128.14
Level III	Sample 2	5	23	1	2	4.48E+06	3.06E+06	1.4669	1268.5	129.11
Level III	Sample 2	5	23	2	1	4.39E+06	3.06E+06	1.4361	1256.8	127.92
Level III	Sample 2	5	23	2	2	4.46E+06	3.08E+06	1.4495	1268.5	129.11
Level III	Sample 3	11	54	1	1	4.85E+06	4.05E+06	1.1982	1238.0	126.01
Level III	Sample 3	11	54	1	2	5.01E+06	4.19E+06	1.1956	1235.3	125.74
Level III	Sample 3	11	54	2	1	4.82E+06	4.07E+06	1.1853	1233.1	125.51
Level III	Sample 3	11	54	2	2	4.99E+06	4.20E+06	1.1881	1236.1	125.81
Level III	Sample 4	11	79	1	1	4.93E+06	4.04E+06	1.2181	1232.3	125.42
Level III	Sample 4	11	79	1	2	5.10E+06	4.18E+06	1.2186	1232.7	125.47
Level III	Sample 4	11	79	2	1	4.86E+06	4.07E+06	1.1935	1231.9	125.39
Level III	Sample 4	11	79	2	2	5.02E+06	4.17E+06	1.2021	1240.8	126.30
Level III	Sample 5	14	7	1	1	4.70E+06	3.87E+06	1.2172	1241.7	126.38
Level III	Sample 5	14	7	1	2	4.77E+06	3.93E+06	1.2157	1240.2	126.24
Level III	Sample 5	14	7	2	1	4.71E+06	3.80E+06	1.2394	1239.6	126.18
Level III	Sample 5	14	7	2	2	4.70E+06	3.83E+06	1.2271	1227.3	124.92
Level III	Sample 6	14	55	1	1	4.62E+06	3.82E+06	1.2092	1233.8	125.58
Level III	Sample 6	14	55	1	2	4.70E+06	3.86E+06	1.2160	1240.7	126.28
Level III	Sample 6	14	55	2	1	4.70E+06	3.94E+06	1.1929	1238.3	126.04
Level III	Sample 6	14	55	2	2	4.60E+06	3.83E+06	1.2030	1248.8	127.10
Level III	Sample 7	19	20	1	1	4.17E+06	3.43E+06	1.2151	1256.2	127.87
Level III	Sample 7	19	20	1	2	4.20E+06	3.45E+06	1.2195	1260.8	128.33
Level III	Sample 7	19	20	2	1	4.24E+06	3.44E+06	1.2321	1257.4	127.98
Level III	Sample 7	19	20	2	2	4.24E+06	3.40E+06	1.2471	1272.7	129.54
Level III	Sample 8	19	65	1	1	4.21E+06	3.45E+06	1.2211	1249.3	127.16
Level III	Sample 8	19	65	1	2	4.23E+06	3.44E+06	1.2299	1258.4	128.08
Level III	Sample 8	19	65	2	1	4.18E+06	3.47E+06	1.2054	1252.0	127.43
Level III	Sample 8	19	65	2	2	4.17E+06	3.44E+06	1.2121	1258.8	128.13
Level III	Sample 9	25	32	1	1	4.83E+06	4.05E+06	1.1932	1237.1	125.92
Level III	Sample 9	25	32	1	2	5.00E+06	4.18E+06	1.1961	1240.1	126.22
Level III	Sample 9	25	32	2	1	4.95E+06	4.11E+06	1.2040	1235.6	125.76
Level III	Sample 9	25	32	2	2	5.08E+06	4.23E+06	1.2028	1234.3	125.63
Level III	Sample 10	25	76	1	1	4.29E+06	3.00E+06	1.4303	1257.0	127.95
Level III	Sample 10	25	76	1	2	4.44E+06	3.09E+06	1.4407	1266.2	128.88
Level III	Sample 10	25	76	2	1	4.38E+06	3.03E+06	1.4453	1283.3	130.61
Level III	Sample 10	25	76	2	2	4.48E+06	3.10E+06	1.4452	1265.2	128.77
Level III	Sample 11	30	16	1	1	4.62E+06	3.80E+06	1.2170	1251.7	127.41
Level III	Sample 11	30	16	1	2	4.64E+06	3.82E+06	1.2162	1251.0	127.33
Level III	Sample 11	30	16	2	1	4.74E+06	3.87E+06	1.2229	1255.1	127.75
Level III	Sample 11	30	16	2	2	4.66E+06	3.86E+06	1.2069	1238.6	126.07
Level III	Sample 12	30	45	1	1	4.22E+06	3.47E+06	1.2155	1253.6	127.59
Level III	Sample 12	30	45	1	2	4.17E+06	3.44E+06	1.2121	1250.1	127.24
Level III	Sample 12	30	45	2	1	4.33E+06	3.49E+06	1.2421	1254.6	127.70
Level III	Sample 12	30	45	2	2	4.29E+06	3.42E+06	1.2552	1267.8	129.04
Level IV	Sample 1	5	11	1	1	4.75E+06	3.14E+06	1.5100	1294.5	131.45
Level IV	Sample 1	5	11	1	2	4.85E+06	3.19E+06	1.5212	1304.1	132.43
Level IV	Sample 1	5	11	2	1	4.79E+06	3.17E+06	1.5127	1290.6	131.06
Level IV	Sample 1	5	11	2	2	4.85E+06	3.19E+06	1.5182	1295.2	131.53
Level IV	Sample 2	5	23	1	1	4.71E+06	3.13E+06	1.5026	1297.1	131.72
Level IV	Sample 2	5	23	1	2	4.82E+06	3.18E+06	1.5132	1306.2	132.64
Level IV	Sample 2	5	23	2	1	4.75E+06	3.13E+06	1.5149	1303.3	132.34
Level IV	Sample 2	5	23	2	2	4.81E+06	3.20E+06	1.5052	1294.9	131.50
Level IV	Sample 3	11	54	1	1	5.32E+06	4.31E+06	1.2341	1290.2	131.02
Level IV	Sample 3	11	54	1	2	5.46E+06	4.43E+06	1.2308	1286.7	130.67
Level IV	Sample 3	11	54	2	1	5.14E+06	4.12E+06	1.2488	1278.3	129.81
Level IV	Sample 3	11	54	2	2	5.28E+06	4.23E+06	1.2486	1278.0	129.78
Level IV	Sample 4	11	79	1	1	5.12E+06	4.12E+06	1.2436	1276.1	129.59
Level IV	Sample 4	11	79	1	2	5.29E+06	4.26E+06	1.2424	1274.8	129.46
Level IV	Sample 4	11	79	2	1	5.03E+06	4.10E+06	1.2274	1278.4	129.82
Level IV	Sample 4	11	79	2	2	5.21E+06	4.26E+06	1.2247	1275.6	129.54
Level IV	Sample 5	14	7	1	1	4.77E+06	3.88E+06	1.2311	1263.7	128.33
Level IV	Sample 5	14	7	1	2	4.86E+06	3.87E+06	1.2564	1289.8	130.98
Level IV	Sample 5	14	7	2	1	4.85E+06	3.80E+06	1.2769	1290.3	131.03
Level IV	Sample 5	14	7	2	2	4.86E+06	3.88E+06	1.2543	1267.5	128.72
Level IV	Sample 6	14	55	1	1	4.83E+06	3.79E+06	1.2747	1295.4	131.54
Level IV	Sample 6	14	55	1	2	4.89E+06	3.87E+06	1.2643	1284.8	130.47
Level IV	Sample 6	14	55	2	1	4.78E+06	3.78E+06	1.2652	1282.3	130.22
Level IV	Sample 6	14	55	2	2	4.85E+06	3.88E+06	1.2512	1268.1	128.78
Level IV	Sample 7	19	20	1	1	4.48E+06	3.57E+06	1.2575	1300.7	132.09
Level IV	Sample 7	19	20	1	2	4.49E+06	3.59E+06	1.2517	1294.7	131.48

Level IV	Sample 7	19	20	2	1	4.43E+06	3.55E+06	1.2492	1293.1	131.31
Level IV	Sample 7	19	20	2	2	4.50E+06	3.61E+06	1.2464	1290.3	131.02
Level IV	Sample 8	19	65	1	1	4.45E+06	3.55E+06	1.2525	1292.5	131.25
Level IV	Sample 8	19	65	1	2	4.48E+06	3.56E+06	1.2594	1299.5	131.97
Level IV	Sample 8	19	65	2	1	4.41E+06	3.51E+06	1.2564	1304.8	132.50
Level IV	Sample 8	19	65	2	2	4.45E+06	3.54E+06	1.2599	1308.4	132.87
Level IV	Sample 9	25	32	1	1	5.08E+06	4.14E+06	1.2253	1279.1	129.89
Level IV	Sample 9	25	32	1	2	5.24E+06	4.27E+06	1.2270	1280.9	130.07
Level IV	Sample 9	25	32	2	1	5.07E+06	4.17E+06	1.2164	1275.9	129.56
Level IV	Sample 9	25	32	2	2	5.23E+06	4.31E+06	1.2144	1273.8	129.36
Level IV	Sample 10	25	76	1	1	4.77E+06	3.18E+06	1.4994	1292.1	131.21
Level IV	Sample 10	25	76	1	2	4.77E+06	3.15E+06	1.5133	1304.1	132.43
Level IV	Sample 10	25	76	2	1	4.78E+06	3.16E+06	1.5103	1299.5	131.97
Level IV	Sample 10	25	76	2	2	4.82E+06	3.19E+06	1.5124	1301.3	132.15
Level IV	Sample 11	30	16	1	1	4.85E+06	3.82E+06	1.2696	1293.9	131.39
Level IV	Sample 11	30	16	1	2	4.81E+06	3.83E+06	1.2571	1281.1	130.10
Level IV	Sample 11	30	16	2	1	4.80E+06	3.83E+06	1.2539	1285.2	130.51
Level IV	Sample 11	30	16	2	2	4.75E+06	3.82E+06	1.2446	1275.6	129.54
Level IV	Sample 12	30	45	1	1	4.42E+06	3.53E+06	1.2539	1291.7	131.17
Level IV	Sample 12	30	45	1	2	4.49E+06	3.58E+06	1.2556	1293.4	131.35
Level IV	Sample 12	30	45	2	1	4.39E+06	3.54E+06	1.2392	1298.3	131.84
Level IV	Sample 12	30	45	2	2	4.47E+06	3.64E+06	1.2267	1285.3	130.52

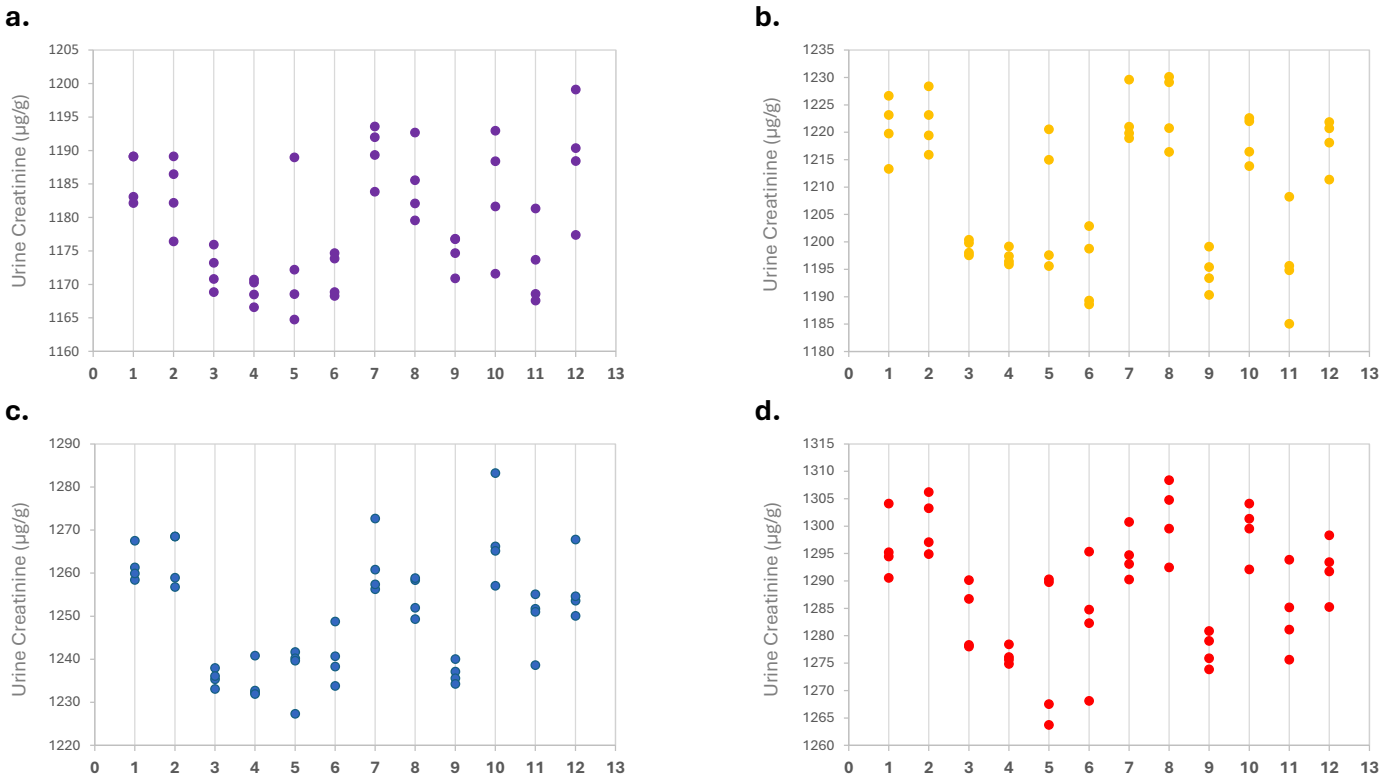

Fig. E2. Plot of relative vial fill order from stratified sampling scheme versus measured creatinine value for SRM 3666 Level I (a), Level II (b), Level III (c), and Level IV (d).

Appendix F. Stability Assessment of SRM 3666

This section contains supplemental information to support the stability assessment of urine albumin and creatinine in SRM 3666 (Level I to Level IV).

F.1. Urine Albumin Stability Assessment

The urine albumin stability assessment was a 7-day study (short-term) to determine the impact of potential shipping temperature conditions encountered during normal shipment of SRM 3666 from NIST to the end-user on the integrity of the endogenous albumin content. Using the NIST RMP for albumin [1], the albumin content of SRM 3666 (Level I to Level IV) was assessed over four temperatures (-80 ºC, -20 ºC, 5 ºC, and 25 ºC) at three timepoints (7-day, 3-day, and 1-day). A total of twelve (12) vials per level of SRM 3666 were randomly selected from the lot (31 boxes, 2500 vials) via a stratified random sampling scheme. See Tables F1 and F2 for the box and vial number for each selected vial. Analysis of the processed stability samples was randomized, sequence generated using random permutation in Excel, to avoid influence of systematic bias on the output measurements and four (4) technical replicate measurements were acquired for each stability sample. The consensus mass concentration values for each condition are shown in Table F3.

Table F1. Sampling scheme for stability assessment of albumin in SRM 3666 (Level I to Level IV).

SRM 3666 Level Number	Box Number	Vial Number	Stability Sample Code
Level I	14	1	1.14.1
Level I	20	19	1.20.19
Level I	14	2	1.14.2
Level I	20	20	1.20.20
Level I	14	5	1.14.5
Level I	20	23	1.20.23
Level I	14	12	1.14.12
Level I	20	30	1.20.30
Level I	14	3	1.14.3
Level I	20	21	1.20.21
Level I	14	10	1.14.10
Level I	20	28	1.20.28
Level I	14	13	1.14.13
Level I	20	31	1.20.31
Level I	14	4	1.14.4
Level I	20	22	1.20.22
Level I	14	11	1.14.11
Level I	20	29	1.20.29
Level I	14	14	1.14.14
Level I	20	32	1.20.32
Level II	14	1	2.14.1
Level II	20	19	2.20.19
Level II	14	2	2.14.2
Level II	20	20	2.20.20
Level II	14	5	2.14.5
Level II	20	23	2.20.23
Level II	14	8	2.14.8
Level II	20	30	2.20.30
Level II	14	3	2.14.3

SRM 3666 Level Number	Box Number	Vial Number	Stability Sample Code
Level II	20	21	2.20.21
Level II	14	6	2.14.6
Level II	20	28	2.20.28
Level II	14	9	2.14.9
Level II	20	31	2.20.31
Level II	14	4	2.14.4
Level II	20	22	2.20.22
Level II	14	7	2.14.7
Level II	20	29	2.20.29
Level II	14	10	2.14.10
Level II	20	32	2.20.32
Level III	14	5	3.14.5
Level III	20	18	3.20.18
Level III	14	6	3.14.6
Level III	20	19	3.20.19
Level III	14	9	3.14.9
Level III	20	22	3.20.22
Level III	14	16	3.14.16
Level III	20	25	3.20.25
Level III	14	7	3.14.7
Level III	20	20	3.20.20
Level III	14	14	3.14.14
Level III	20	23	3.20.23
Level III	14	17	3.14.17
Level III	20	26	3.20.26
Level III	14	8	3.14.8
Level III	20	21	3.20.21
Level III	14	15	3.14.15
Level III	20	24	3.20.24
Level III	14	18	3.14.18
Level III	20	27	3.20.27
Level IV	14	21	4.14.21
Level IV	20	72	4.20.72
Level IV	14	22	4.14.22
Level IV	20	73	4.20.73
Level IV	14	25	4.14.25
Level IV	20	76	4.20.76
Level IV	14	28	4.14.28
Level IV	20	79	4.20.79
Level IV	14	23	4.14.23
Level IV	20	74	4.20.74
Level IV	14	26	4.14.26
Level IV	20	77	4.20.77
Level IV	14	29	4.14.29
Level IV	20	80	4.20.80
Level IV	14	24	4.14.24
Level IV	20	75	4.20.75
Level IV	14	27	4.14.27
Level IV	20	78	4.20.78
Level IV	14	30	4.14.30
Level IV	20	81	4.20.81

Table F2. Time-temperature combinations for each vial of SRM 3666 (Level I to Level IV) selected for the stability assessment.

SRM 3666 Level Number	Stability Sample Code	Temperature (°C)	Time (day)
Level I	1.14.1	-80	7
Level I	1.20.19	-80	7
Level I	1.14.2	-20	7
Level I	1.20.20	-20	7
Level I	1.14.5	5	7
Level I	1.20.23	5	7
Level I	1.14.12	25	7
Level I	1.20.30	25	7
Level I	1.14.3	-20	3
Level I	1.20.21	-20	3
Level I	1.14.10	5	3
Level I	1.20.28	5	3
Level I	1.14.13	25	3
Level I	1.20.31	25	3
Level I	1.14.4	-20	1
Level I	1.20.22	-20	1
Level I	1.14.11	5	1
Level I	1.20.29	5	1
Level I	1.14.14	25	1
Level I	1.20.32	25	1
Level II	2.14.1	-80	7
Level II	2.20.19	-80	7
Level II	2.14.2	-20	7
Level II	2.20.20	-20	7
Level II	2.14.5	5	7
Level II	2.20.23	5	7
Level II	2.14.8	25	7
Level II	2.20.30	25	7
Level II	2.14.3	-20	3
Level II	2.20.21	-20	3
Level II	2.14.6	5	3
Level II	2.20.28	5	3
Level II	2.14.9	25	3
Level II	2.20.31	25	3
Level II	2.14.4	-20	1
Level II	2.20.22	-20	1
Level II	2.14.7	5	1
Level II	2.20.29	5	1
Level II	2.14.10	25	1
Level II	2.20.32	25	1
Level III	3.14.5	-80	7
Level III	3.20.18	-80	7
Level III	3.14.6	-20	7
Level III	3.20.19	-20	7
Level III	3.14.9	5	7
Level III	3.20.22	5	7
Level III	3.14.16	25	7
Level III	3.20.25	25	7
Level III	3.14.7	-20	3
Level III	3.20.20	-20	3
Level III	3.14.14	5	3
Level III	3.20.23	5	3
Level III	3.14.17	25	3
Level III	3.20.26	25	3
Level III	3.14.8	-20	1

SRM 3666 Level Number	Stability Sample Code	Temperature (°C)	Time (day)
Level III	3.20.21	-20	1
Level III	3.14.15	5	1
Level III	3.20.24	5	1
Level III	3.14.18	25	1
Level III	3.20.27	25	1
Level IV	4.14.21	-80	7
Level IV	4.20.72	-80	7
Level IV	4.14.22	-20	7
Level IV	4.20.73	-20	7
Level IV	4.14.25	5	7
Level IV	4.20.76	5	7
Level IV	4.14.28	25	7
Level IV	4.20.79	25	7
Level IV	4.14.23	-20	3
Level IV	4.20.74	-20	3
Level IV	4.14.26	5	3
Level IV	4.20.77	5	3
Level IV	4.14.29	25	3
Level IV	4.20.80	25	3
Level IV	4.14.24	-20	1
Level IV	4.20.75	-20	1
Level IV	4.14.27	5	1
Level IV	4.20.78	5	1
Level IV	4.14.30	25	1
Level IV	4.20.81	25	1

Table F3. Stability study mass concentration (mg/L) values for albumin in SRM 3666 (Level I to Level IV). (Mass concentration value - $\bar{\bar{X}}_{mg/L}$; uncertainty of mass concentration value - $u_{mg/L}$)

		SRM 3666 Level I		SRM 3666 Level II		SRM 3666 Level III		SRM 3666 Level IV	
Day	**Temp. (°C)**	$\bar{\bar{X}}_{mg/L}$	$u_{mg/L}$	$\bar{\bar{X}}_{mg/L}$	$u_{mg/L}$	$\bar{\bar{X}}_{mg/L}$	$u_{mg/L}$	$\bar{\bar{X}}_{mg/L}$	$u_{mg/L}$
1	**-20**	8.35	0.82	34.54	0.67	105.21	4.10	351.72	31.34
1	**5**	8.38	0.70	33.68	0.60	109.10	4.38	350.11	27.78
1	**25**	8.25	0.77	32.91	0.61	109.72	7.71	354.51	27.65
3	**-20**	8.54	0.72	33.20	0.60	104.03	3.75	366.96	24.37
3	**5**	8.61	0.85	33.59	0.72	103.08	4.18	378.83	26.72
3	**25**	8.10	0.58	32.73	0.94	100.07	4.80	359.45	31.62
7	**-80**	8.59	0.64	33.53	0.54	104.82	4.52	377.85	25.18
7	**-20**	8.56	0.61	32.03	0.90	98.15	4.02	365.97	25.10
7	**5**	8.43	0.69	31.54	1.03	103.22	4.50	372.33	24.33
7	**25**	8.26	0.63	30.63	0.99	99.50	4.08	369.47	27.28

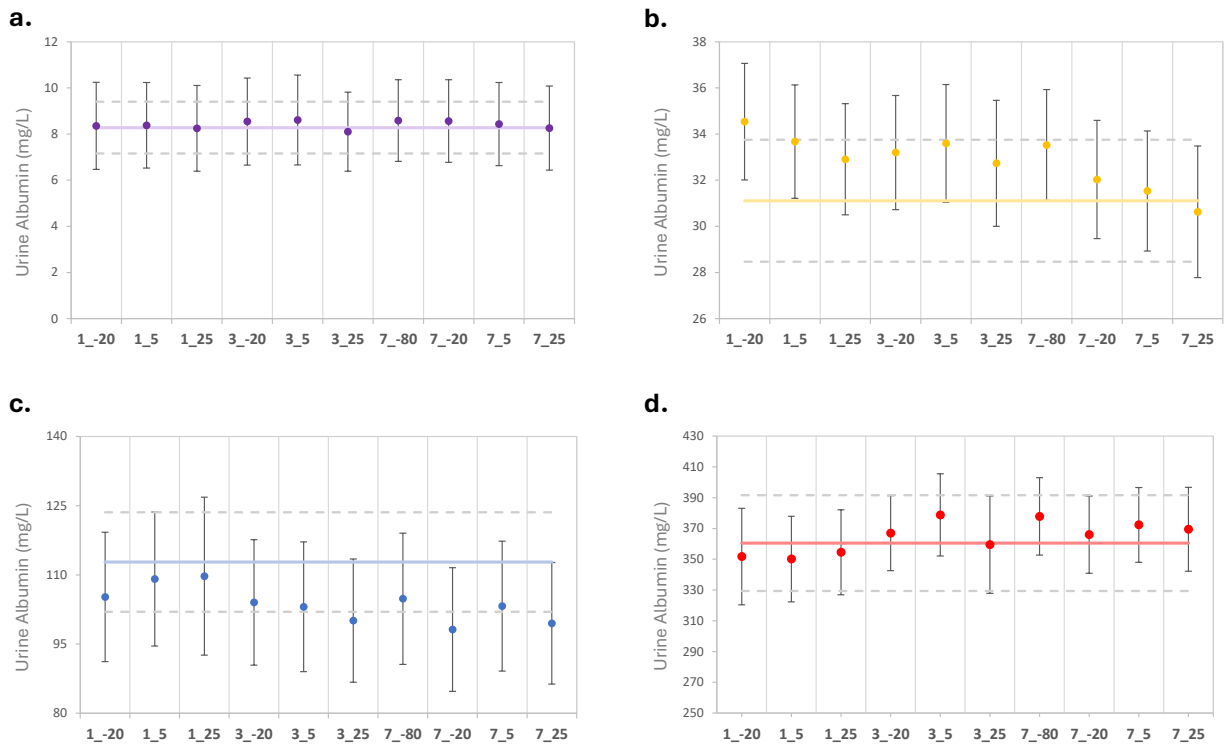

Fig. F1. Plots of sample number (vial number) versus measured endogenous albumin content (mg/L) for SRM 3666 Level I (a), Level II (b), Level III (c), and Level IV (d). The error bars represent the expanded uncertainty (mg/L, k=2) of the endogenous albumin content in SRM 3666 Level I to Level IV (n = 20) observed for each stability sample. The dotted gray lines represent the expanded uncertainty interval of the certified value and the solid lines (purple, yellow, blue, red) represent the certified value for each level (Table 3). (**1_-20**: 1-day, -20 °C; **1_5**: 1-day, 5 °C; **1_25**: 1-day, 25 °C; **3_-20**: 3-day, -20 °C; **3_5**: 3-day, 5 °C; **3_25**: 3-day, 25 °C; **7_-80**: 7-day, -80 °C; **7_-20**: 7-day, -20 °C; **7_5**: 7-day, 5 °C; **7_25**: 7-day, 25 °C)

F.2. Urine Creatinine Stability Assessment

The long-term stability of urine creatinine was evaluated through two independent monitoring studies conducted at approximately one- and two-year intervals following the 2023 release date of SRM 3666. These longitudinal assessments comprised a Commutability Assessment in 2024 [30] and an Extent-of-Equivalence Assessment in 2025 [31], both designed to verify the stability of the certified values under the specified storage conditions. All measurements were performed using the modified-NIST RMP [21, 22]. To reduce systematic bias, analysis of the stability samples in both studies was randomized, with process and technical replicates. The resulting mass concentration values for urine creatinine were within the confidence interval of the certified value ($\bar{\bar{x}}_{mg/dL} \pm U_{mg/dL}$), demonstrating no significant degradation over the monitoring period. The material remains stable under the specified storage conditions and remains fit for its intended use. The consensus mass concentration values ($\bar{\bar{x}}_{mg/dL}$) and associated expanded uncertainties ($U_{mg/dL}$; k = 2.201) are summarized in Table F4, while the long-term stability profile is visually represented in Figure F2.

Table F4. Stability study mass concentration (mg/L) values for urine creatinine in SRM 3666 (Level I to Level IV).

Stability Study	SRM 3666 Level	Stability Consensus Mass Concentration Value (mg/dL)	Stability Expanded Uncertainty - Consensus Mass Concentration Value (mg/dL)	Certified Mass Concentration Value (mg/dL) (Table 4)	Certified Expanded Uncertainty Mass Concentration Value (mg/dL) (Table 4)
Commutability	**Level I**	116.37	2.54	119.69	2.63
Commutability	**Level II**	119.37	2.60	122.65	2.73
Commutability	**Level III**	129.89	2.81	127.19	2.78
Commutability	**Level IV**	127.15	2.81	130.90	2.96
Extent of Equivalence	**Level I**	115.46	4.81	119.69	2.63
Extent of Equivalence	**Level II**	116.50	7.33	122.65	2.73
Extent of Equivalence	**Level III**	123.25	11.56	127.19	2.78
Extent of Equivalence	**Level IV**	127.01	3.74	130.90	2.96

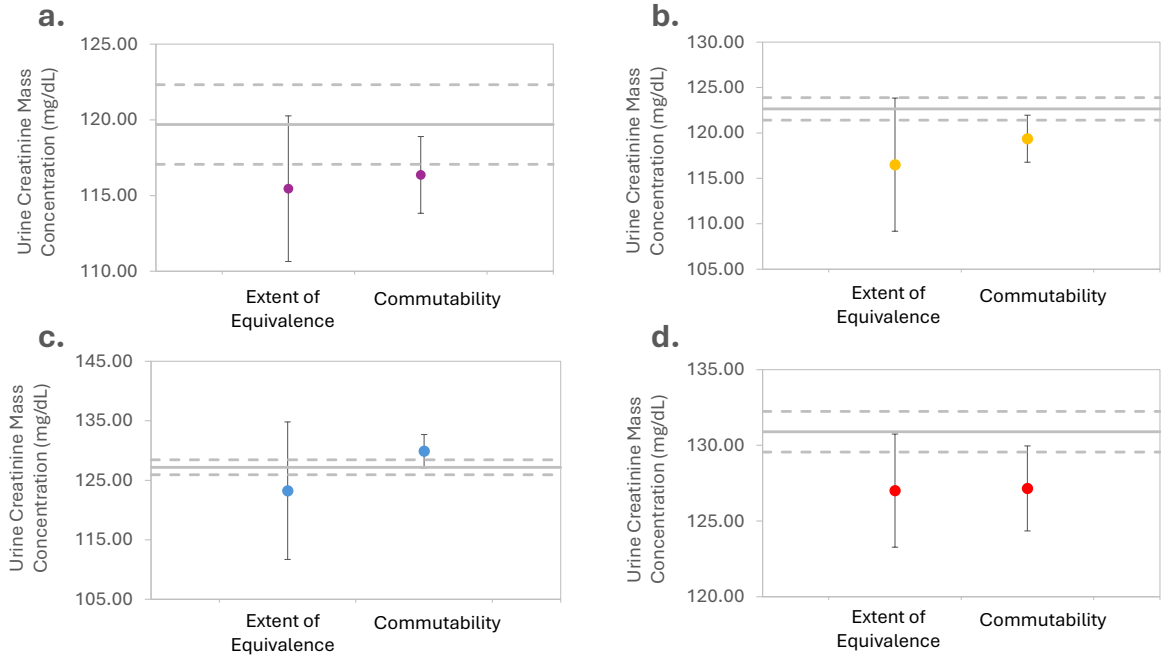

Fig. F2. Plots of stability study versus measured urine creatinine content (mg/dL) for SRM 3666 Level I (a), Level II (b), Level III (c), and Level IV (d). The error bars represent the expanded uncertainty (mg/dL, k=2.201) of the urine creatinine content in SRM 3666 Level I to Level IV (n = 9) observed for each stability study. The dotted gray lines represent the expanded uncertainty interval of the certified value and the solid gray lines represent the certified value for each level.

Appendix G. Commutability Assessment of SRM 3666

Commutability studies of urine albumin and creatinine in SRM 3666 (Level I to Level IV) were conducted by analyzing single-donor human urine samples and the SRM using the NIST RMPs [13-15,20-22] and IVD-MPs (immunoturbidimetric, Enzymatic, and Jaffé methods). The commutability of SRM 3666 (Level I to Level IV) was assessed using the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) approach (bracketed) for IVD-MPs from six major IVD manufacturers (Abbott Laboratories, Beckman Coulter, Mindray, QuidelOrtho, Roche Diagnostics, Siemens Healthcare Diagnostics) [30]. The commutability criterion, represented by the maximum allowable noncommutability bias (MANCB), for urine albumin for Level I was $MANCB_I$ 12.9 % and for Level II to Level IV was $MANCB_{II\ to\ IV}$ 9.9 % [30]. The commutability criterion, represented by MANCB, for urine creatinine (Enzymatic and Jaffé) for Level I to Level IV was MANCB 9.3 % [30]. The commutability profile for urine albumin exhibited variability among the four levels of SRM 3666, as shown in Table G1. The commutability profile for urine creatinine is summarized in Table G2. The results demonstrated satisfactory commutability of urine albumin and creatinine in SRM 3666 (Level I to Level IV) for the evaluated IVD-MPs, which suggests that SRM 3666 is suitable for use as a secondary measurement standard in the calibration hierarchy for urine albumin and creatinine for select IVD-MPs. A full description of the commutability assessment is provided in Ref. [30].

Table G1. Summary commutability matrix for SRM 3666 (Level I to Level IV) for urine albumin IVD-MPs with $MANCB_I$ = 12.9 % (Level I) and $MANCB_{II\ to\ IV}$ = 9.9 % (Level II to Level IV).

IVD-MP	NIST RMP for Urine Albumin - Bracketed[a]			
	Level I	Level II	Level III	Level IV
Abbott Alinity c	C	C	C	C
Beckman Coulter DxC 700 AU	C	C	C	C
Mindray BS-800M	C	C	C	
QuidelOrtho VITROS 5600	C	C	C	
Roche Cobas Pro c503	C	[I]	[I]	C
Siemens Atellica CH Microalbumin – µALB_2 (Reagent 1)	I	[I]	[I]	I
Siemens Atellica CH Microalbumin – UAlb (Reagent 2)	I	[I]	[I]	[I]
Siemens BN ProSpec		C	C	
Siemens Dimension EXL	I	C	C	

[a] C, Commutable; I, Inconclusive. For an Inconclusive (I) commutability conclusion: I indicates that the d_{RM} value is within ± MANCB ($MANCB_I$ = ± 12.9 %; $MANCB_{II\ to\ IV}$ = ± 9.9 %) and $U(d_{RM})$ overlaps ± MANCB by more than 25 %; [I] indicates that the d_{RM} value is outside ± MANCB (± 12.9 %; ± 9.9 %) and $U(d_{RM})$ overlaps ± MANCB. Blank gray cells indicate that the level and associated clinical samples were not analyzed because the concentration in the SRM Level was outside the measuring interval for the analyzer.

Table G2. Summary commutability matrix for SRM 3666 (Level I to Level IV) for urine creatinine (Enzymatic and Jaffé) clinical measurement procedures with MANCB = 9.3 %.

IVD-MP	NIST modified-RMP for Urine Creatinine (Enzymatic) – Bracketed[a]				NIST modified-RMP for Urine Creatinine (Jaffé) – Bracketed[a]			
	Level I	Level II	Level III	Level IV	Level I	Level II	Level III	Level IV
Abbott Alinity c	C	C	C	C	C	C	C	C
Beckman Coulter DxC 700 AU	C	C	C	C	C	C	C	C
Mindray BS-800M	C	C	C	C				
QuidelOrtho VITROS 5600	C	C	C	C				
Roche Cobas Pro c503	C	C	C	C	C	C	C	C
Siemens Atellica CH Enzymatic Creatinine	C	C	C	C				
Siemens Atellica CH Creatinine_2 (Jaffé Reagent 1)					C	C	C	C
Siemens Atellica CH Creatinine_3 (Jaffé Reagent 2)					C	C	I*	C
Siemens Dimension EXL	C	C	C	C	C	C	C	C

[a] C, Commutable; I, Inconclusive. For an Inconclusive (I) commutability conclusion: I* indicates that the d_{RM} value is within MANCB confidence interval (MANCB = ± 9.3 %) and $U(d_{RM})$ overlaps ± MANCB by less than 25 %. Blank gray cells indicate that the MPs were not applicable to the analyzers. No urine creatinine-Jaffé MP is available for the Mindray BS-800M and Ortho VITROS 5600 analyzers.

Appendix H. List of Symbols, Abbreviations, and Acronyms

ACR	albumin-to-creatinine ratio
AMBIC	ammonium bicarbonate
CKD	chronic kidney disease
COA	certificate of analysis
DTT	dithiothreitol
ELISA	enzyme-linked immunosorbent assay
HCl	hydrochloric
HSA	human serum albumin
IAM	iodoacetamide
ID-LC-MS/MS	isotope dilution-liquid chromatography-tandem mass spectrometry
IFCC	International Federation of Clinical Chemistry
IVD	in vitro diagnostic
IVD-MP	in vitro diagnostic – measurement procedure
JCTLM	Joint Committee for Traceability in Laboratory Medicine
LC-MS	liquid chromatography-mass spectrometry
MRM	multiple reaction monitoring
NHANES	National Health and Nutrition Examination Survey
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NIDDK LWG	NIDDK Laboratory Working Group
NIST	National Institute of Standards and Technology
PDVF	polyvinylidene fluoride
qt-MRM	quantitative MRM transition
*r*HSA	recombinant human serum albumin
RMP	reference measurement procedure
RRF	relative response factor
SED	NIST Statistical Engineering Division
SIM	selected ion monitoring
SRM	Standard Reference Material®
VCU	Virginia Commonwealth University
WG-SAU	IFCC Working Group for the Standardization of Albumin Assays in Urine

Appendix I. Change Log

Corrections made in the errata update do not alter existing or introduce substantive technical information but rather are intended to remove ambiguity and improve interpretation of the work.

Page Number	Update Description
ii	Add “Laboratory Working Group”
2	Add “Laboratory Working Group (LWG)”
5	Add “The Abbott Architect Enzymatic Creatinine Assay is a colorimetric method that is based on enzymatic principle to determine creatinine content in human urine. Creatinine in the sample is hydrolyzed by creatininase to form creatine and creatine is hydrolyzed by creatinase to form sarcosine and urea. The sarcosine produced from this reaction is then oxidized by sarcosine oxidase to produce glycine, formaldehyde, and hydrogen peroxide. The hydrogen peroxide produced from this reaction reacts with 4-aminoantipyrine and N-ethyl-N-sulfopropyl-m-toluidine in the presence of peroxidase to yield a quinoneimine dye. The change in absorbance is directly proportional to the concentration of creatinine in the sample.”
7	Table 3, replace “g/mL” with “mg/L”
11	Remove “standard uncertainty”
14	Table A3, replace “Abbott Architect Creatinine Assay - Jaffee” with “Abbott Architect Enzymatic Creatinine Assay”
14	Replace “Jaffee” with “enzymatic”
14	Table A1, replace “Abbott Albumin / Abbott Creatinine Assays” with “Abbott Architect Microalbumin / Abbott Architect Enzymatic Creatinine Assays”
16	Table C2, replace “Standard Deviation” with “Combined Standard Uncertainty”
20	Table D1, replace “Standard Deviation” with “Combined Standard Uncertainty”
20	Table D1, match Certified Values for Creatinine to values in Table 4 (pg. 10)
22	Add “NIDDK Laboratory Working Group (LWG)” to Appendix E
11	Text added to Section 4.0
11	Text added to Section 5.0
29	Added Appendices G, H, and I
	Added new references and updated reference numbers in text
	Added author
	Added new reference (ref. 31)
8-9	Added uncertainty equations for urine albumin
10-12	Added measurement and uncertainty equations for urine creatinine
13, 35-36	Added long-term stability data for urine creatinine