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Extent-of-Equivalence Assessment of Certified Reference Materials for Albumin and Creatinine in Human Urine

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Abstract

Accurate urine albumin test results are crucial for diagnosing and managing kidney disease. To ensure accuracy and consistency, clinical lab results need to be standardized. This can be achieved by establishing a calibration hierarchy that links clinical results to the foundational unit of measure, the International System of Units (SI). The National Institute of Standards and Technology (NIST) and the Health Sciences Authority (HSA), Singapore, have developed two certified reference materials (CRMs) for albumin and creatinine in human urine. An extent-of-equivalence assessment was performed to compare the certified values of urine albumin and creatinine in the two human urine CRMs (NIST SRM 3666 and HRM-3004A). The overlap observed in the equivalence assessment between the measured $[X_i \pm U_{95}(X_i)]$ and certified $[C_i \pm U_{95}(C_i)]$ intervals indicate that the intervals agree within their assigned uncertainties with about a 95 % level of confidence. Therefore, the CRMs were deemed equivalent at a 95 % level of confidence for measuring urine albumin and creatinine. The equivalence of these CRMs ensures that their interchangeable use in a calibration hierarchy does not introduce additional bias, thereby maintaining the integrity of the calibration hierarchy and ensuring that measurement results remain reliable and without significant impacts on uncertainty or quality. By establishing a robust calibration hierarchy for urine albumin and creatinine measurements, we can enhance the reliability and accuracy of clinical laboratory measurements for kidney disease diagnosis and monitoring, ultimately leading to better patient outcomes and more effective healthcare management.

Keywords

Albumin, Calibration Hierarchy, Certified Reference Material (CRM), Creatinine, Extent-of-equivalence, Metrological Traceability, Standard Reference Material (SRM).

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Author Contributions

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EXECUTIVE SUMMARY

Kidney disease is a significant global health concern that requires accurate diagnosis and monitoring for effective management. Urine albumin is a key biomarker for kidney disease, and its accurate measurement is critical for informed decision-making by healthcare practitioners. To ensure uniformity and consistency in clinical laboratory results, standardization is essential. This can be achieved by establishing a calibration hierarchy that links clinical results to higher-order reference materials and reference measurement procedures, thereby ensuring metrological traceability. The National Institute of Standards and Technology (NIST) and other metrology organizations play a crucial role in developing and maintaining these higher-order reference materials and measurement procedures. By establishing metrological traceability, clinical laboratory results can be made comparable across different laboratories and methods, facilitating the comparison of clinical data and the development of standardized clinical guidelines. Ultimately, the standardization of clinical urine albumin results will enhance the confidence and global comparability of clinical results for kidney disease, leading to improved patient care and outcomes.

1. INTRODUCTION

Urine albumin is a major diagnostic and prognostic biomarker for kidney disease, and is used for clinical decisions associated with renal therapy. The accuracy and consistency of urine albumin results significantly influence the decisions made by healthcare practitioners regarding the diagnosis and treatment of kidney disease. Therefore, it is crucial for healthcare practitioners to have access to reliable and comparable patient results, which can be achieved through the standardization of clinical laboratory results for urine albumin. When clinical urine albumin results are standardized, the clinical value is equivalent and independent of the method or laboratory used. Standardization of urine albumin clinical results can be achieved by establishing metrological traceability of values assigned to clinical samples to higher-order reference materials and reference measurement procedures [1]. Metrological traceability, as defined in ISO 17511, is the “*property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty*” [1]. The metrological traceability of clinical urine albumin results requires a calibration hierarchy, which consists of a series of value assignments that create a link between clinical results and the foundational unit of measure, the International System of Units (SI). A calibration hierarchy, as defined in ISO 17511, is a “*sequence of calibrations from a reference to the final measuring system, where the outcome of each calibration depends on the outcome of the previous calibration*” [1].

The National Institute of Standards and Technology (NIST) has partnered with the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Laboratory Working Group and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Working Group for the Standardization of Albumin Assays in Urine (WG-SAU) to develop higher-order certified reference materials (CRMs) and a reference measurement procedure (RMP) to establish a calibration hierarchy for clinical urine albumin results. Figure 1 illustrates the CRMs and RMP developed by NIST to support a calibration hierarchy for clinical urine albumin results. The reference materials are indicated as “*m*” and measurement procedures are indicated as “*p*” in Fig. 1. The NIST RMP for Urine Albumin (Fig. 1, *p.3*) is intended for use in the value assignment of albumin in human urine in NIST Standard Reference Material® (SRM) 3666 (Fig. 1, *m.2*), a matrix-based (human urine) secondary measurement standard [2-6]. Calibration of the NIST RMP for Urine Albumin is SI traceable via the use of NIST SRM 2925 as the calibrator [7-8]. NIST SRM 2925, a recombinant solution of albumin, serves as the primary measurement standard (Fig. 1, *m.1*) for the urine albumin calibration hierarchy (Fig. 1). The NIST RMP for Urine Albumin and NIST SRM 2925 are currently listed in the Joint Committee for Traceability in Laboratory Medicine (JCTLM) database for reference measurement procedures [9] and reference materials [10]. NIST SRM 3666 is a four (4)-level (Level I to Level IV) human urine material intended for use as a secondary measurement standard (Fig. 1, *m.2*) to support the accuracy and comparability of clinical urine albumin and creatinine results used in clinical decisions for kidney disease [5-6].

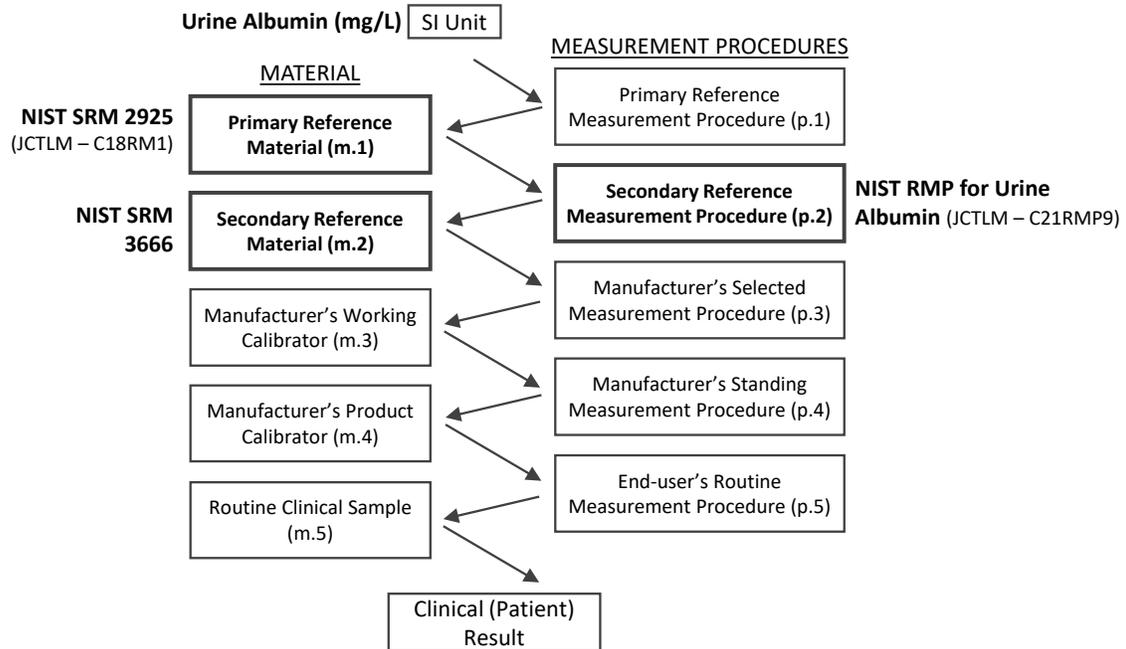


Fig. 1. The proposed calibration hierarchy for clinical urine albumin results using the NIST CRMs (SRM 2925 and SRM 3666) and the NIST RMP for Urine Albumin. Adapted from ISO 17511, Ref. [1].

The four levels of SRM 3666 (Levels I to IV) include certified values for urine albumin and creatinine (Tables 1 and 2).

Table 1. Certified values with associated uncertainties for urine albumin in NIST SRM 3666 (Level I to Level IV) [5].

Level	Certified Mass Fraction (mg/g)	Expanded Uncertainty (mg/g; k = 2)	Certified Mass Concentration (mg/L)	Expanded Uncertainty (mg/L; k = 2)
NIST SRM 3666 Level I	0.008	0.001	8.28	1.12
NIST SRM 3666 Level II	0.031	0.003	31.11	2.64
NIST SRM 3666 Level III	0.111	0.011	112.77	10.79
NIST SRM 3666 Level IV	0.355	0.031	360.50	31.11

Table 2. Certified values with associated uncertainties for urine creatinine in NIST SRM 3666 (Level I to Level IV) [5].

Level	Certified Mass Fraction (µg/g)	Expanded Uncertainty (µg/g; k = 2.201)	Certified Mass Concentration (mg/dL)	Expanded Uncertainty (mg/dL; k = 2.201)
NIST SRM 3666 Level I	1178.96	25.05	119.69	2.63
NIST SRM 3666 Level II	1209.58	26.28	122.65	2.73
NIST SRM 3666 Level III	1249.58	26.37	127.19	2.78
NIST SRM 3666 Level IV	1289.01	27.71	130.90	2.96

A human urine-based CRM for albumin and creatinine produced by the Health Sciences Authority (HSA), Singapore (HRM-3004A), is currently listed in the JCTLM reference material database.

HRM-3004A is a two-level human urine material with certified values for albumin and creatinine (Table 3).

Table 3. Certified values with associated uncertainties for urine albumin and creatinine in HSA HRM-3004A (Level 1 and Level 2) [11].

Level	Level	Urine Albumin Certified Mass Concentration (mg/L)	Expanded Uncertainty (mg/L; k = 2)	Urine Creatinine Certified Concentration (mmol/L)	Expanded Uncertainty (mmol/L; k = 2)
HSA HRM-3004A (STY-0018-053)	Level 1	40.1	2.4	3.57	0.093
HSA HRM-3004A (STY-0018-054)	Level 2	226	11	10.82	0.37

To compare the certified urine creatinine values, the certified unit of mmol/L was converted to mass concentration (mg/dL) using the density at 23 °C (Level 1-1.0068 g/ml and Level 2-1.0167 g/ml) and relative molecular mass of creatinine (113.12 g/mol) [11]. The calculated mass concentration values for the HRM-3004A Level 1 and Level 2 are listed in Table 4.

Table 4. Calculated mass concentration values for urine creatinine in HSA HRM-3004A (Level 1 and Level 2).

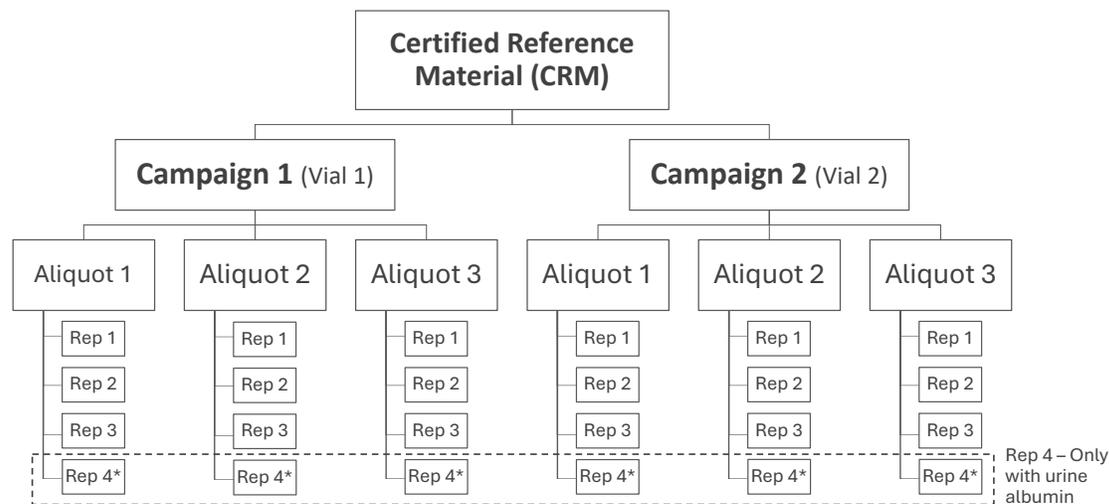
Level	Level	Mass Concentration (mg/dL)	Expanded Uncertainty (mg/dL; k = 2)
HSA HRM-3004A (STY-0018-053)	Level 1	40.35	1.050
HSA HRM-3004A (STY-0018-054)	Level 2	122.40	4.19

An extent-of-equivalence assessment was performed to compare the certified values of urine albumin and creatinine in NIST SRM 3666 (Level I to Level IV) and HSA HRM-3004A. This evaluation is crucial for determining whether the certified values assigned to these CRMs are consistent with their specified uncertainties and suitable for implementation within a calibration hierarchy. By utilizing the NIST RMPs for urine albumin and creatinine [2-4, 12-14], the comparison provides a comprehensive assessment of the equivalence between the two CRMs. The results of this comparison are essential for ensuring that clinical laboratories can rely on either CRM for accurate and consistent measurements, thereby supporting the diagnosis and management of kidney disease.

2. EXPERIMENTAL PROCEDURE

The extent-of-equivalence results for SRM 3666 (Level I to Level IV) and HRM-3004A (Level 1 and Level 2) were obtained using the NIST RMPs for Urine Albumin [2-4, 9] and Creatinine (modified) [12-14]. Two independent vials of each CRM, referred to as Campaign 1 and Campaign 2, were analyzed independently (with separate calibration systems) in the study. For each Campaign (vial), three (3) aliquots (process replicates) were independently processed and analyzed according to the experimental procedures outlined in the NIST RMP for Urine Albumin [2-4, 9] and the NIST RMP for Creatinine [12-14]. The aliquots (process replicates) were analyzed in a randomized sequence with replicate measurements (multiple injections of one preparation separated in time), ensuring that the technical measurements for each aliquot were conducted

independently across separate analytical runs. The randomization of the sequence order and separation of the technical replicates aimed to minimize systematic errors associated with LC-MS analysis of the CRMs. The same analyst prepared all the samples and conducted all the measurements. The experimental design for assessing the extent-of-equivalence of the CRMs is summarized in Fig. 2.



(CRM – Represents NIST SRM 3666 (Level I to Level IV) or HSA HRM-3004A (Level 1 and Level 2); Aliquot - Process Replicate; Rep - Technical Replicates)

Fig. 2. Measurement design of extent-of-equivalence assessment for human urine CRMs.

2.1. URINE ALBUMIN

The equivalence assessment measurements for urine albumin detailed in this section were performed using the NIST RMP for Urine Albumin [2-4, 9]. The albumin measurements were performed at NIST, and the statistical analysis of the data for the determination of albumin was provided by NIST Statistical Engineering Division (SED).

Traceability Statement. Traceability to amount-of-substance units of the SI is based on the certified value of the recombinant human serum albumin reference standard (NIST SRM 2925) and appropriate uncertainties.

Materials. NIST SRM 2925 Recombinant Human Serum Albumin Solution (Primary Reference Calibrator for Urine Albumin) (Frozen) [7-8, 10] was used as a reference standard for albumin and has a certified mass concentration of (0.958 ± 0.022) g/L; NIST SRM 3666 Albumin and Creatinine in Frozen Human Urine [5-6] (Certificate in Appendix C); Health Sciences Authority (HSA), Singapore (HRM-3004A) [11] (Certificate in Appendix D); ^{15}N -labeled recombinant human serum albumin (rHSA) (Albumin Biosciences; Huntsville, AL); ammonium bicarbonate (AMBIC); Trypsin-Gold MS-grade (Promega, Madison, WI, USA); Dithiothreitol (Pierce); Iodoacetamide (Pierce); high-purity LC-MS grade water with 0.1 % (volume fraction) formic acid and acetonitrile with 0.1 % (volume fraction) formic acid (Honeywell Burdick and Jackson).

Experimental Design. The calibration solutions were gravimetrically prepared from the NIST SRM 2925 and isotopically labeled internal standard (IS; ¹⁵N-labeled recombinant HSA) stock solutions in 50 mmol/L ammonium bicarbonate. A bracketed calibration system (bracketed around the SRM 3666 levels) was used to determine the urine albumin content in each sample. The calibration levels and urine albumin concentration ranges for the bracketed calibration approach are outlined in Appendix A, Table A1. The set of aliquots for each campaign of both CRMs were separated into two sets (Campaign Set 1 and Campaign Set 2), as shown in Appendix A, Table A2. Each sample consisted of 130 μL unlabeled albumin material (SRM 2925, SRM 3666, or HRM-3004A) and 10 μL IS. The solutions were allowed to solubilize overnight (approx. 12 h) at 4 °C prior to enzymatic (trypsin) digestion. The following day, the samples were digested with trypsin using the enzymatic digestion protocol outlined in the NIST RMP for Urine Albumin [2-4, 9]. Following equilibration to room temperature (RT, approx. 25 °C), each analysis set (calibrant samples and CRMs) was incubated at 97 °C for 10 min to denature albumin and cooled to RT. The samples were reduced with 5 mmol/L dithiothreitol at 60 °C for 30 min, followed by alkylation with 15 mmol/L iodoacetamide at RT for 30 min in the dark. An approximate 1:30 mass ratio of trypsin-to-total protein was used for digestion, and the digestion reaction was conducted at 37 °C for 24 h. Following digestion, the pH of the samples was reduced with 50 mL/L formic acid in water and incubated for 45 min. at 37 °C to quench the digestion reaction. The samples were concentrated (no heat) overnight and resuspended in 100 μL of 0.1 % (volume fraction) formic acid in water the next day for tandem MS analysis. LC-MS/MS analysis was conducted following the protocol specified in the NIST RMP for Urine Albumin [2-4, 9]. Analysis of the samples was randomized to avoid the influence of systematic bias on the output measurements. The randomized run sequence (generated using random permutation in Excel) for the replicate measurements (4) of each campaign set (Campaign Set 1 and Campaign Set 2) is outlined in Appendix A, Table A3. A total of 23 MRM transitions (unlabeled and IS) were monitored for each sample. All measurements within each campaign were made under repeatability conditions, and the two campaigns were separated in time by 5 days. Data from the five quantitative MRM (qt-MRM) transitions were used in the consensus means analysis to determine the mass fraction consensus value ($\bar{X}_{mg/g}$) of each sample. The density values (Tables 5 and 6) for each CRM were used in the unit conversion from mass fraction (mg/g) to mass concentration (mg/L). The consensus mass concentration values ($\bar{X}_{mg/L}$) for albumin and associated expanded uncertainties (k = 2) were determined by the NIST SED.

Table 5. Certified density values and expanded uncertainty for NIST SRM 3666 (Level I to Level IV) [5].

Level	Density (g/mL)	Expanded Uncertainty (k = 2, g/mL)
NIST SRM 3666 Level I	1.01519	0.00058
NIST SRM 3666 Level II	1.01397	0.00048
NIST SRM 3666 Level III	1.01784	0.00058
NIST SRM 3666 Level IV	1.01549	0.00070

Table 6. Density values for HSA HRM-3004 (Level 1 and Level 2) [11].

Level	Density (g/mL)
HRM-3004A Level 1	1.00680
HRM-3004A Level 2	1.01067

2.2. URINE CREATININE

The equivalence assessment measurements for urine creatinine detailed in this section were performed using a modification of the NIST RMP for Serum Creatinine [12-14]. The creatinine measurements were performed at NIST, and the statistical analysis of the data for the determination of creatinine was provided by NIST SED.

Traceability Statement. Traceability to measurement units of the SI expressed as a mass fraction is based on the certified value of the creatinine reference standard (SRM 914b Creatinine), purity assessment, and appropriate uncertainties.

Materials. NIST SRM 914b Creatinine (mass purity of 99.9 % \pm 0.1 %, Certificate of Analysis); stable isotope labeled IS material (d_3 -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).

Experimental Design. Calibration solutions with known unlabeled-to-labeled creatinine mass ratios were prepared gravimetrically. The IS solutions were added to all samples at the beginning of the sample preparation process. To achieve a mass ratio of approximately 1:1, the amount of IS was adjusted to 70 μ g, corresponding to a sample volume of around 100 μ L. However, for the HSA HRM-3004A Level 1 material, which had a low concentration and limited sample availability, only 40 μ g of IS was used. The final sample volume was adjusted in the last dilution step before LC-MS analysis. The aliquots (3) for each of the two campaigns were divided into two sets (Campaign Set 1 and Campaign Set 2), as detailed in Appendix B, Table B1. The processed samples, including calibration solutions, SRM 3667 quality control solutions, NIST SRM 3666 samples, and HSA HRM-3004A samples, were analyzed using LC-MS (Agilent 1260 Infinity II Series LC system coupled to an Agilent InfinityLab Quadrupole MS).

The LC conditions were isocratic using HPLC-grade water with 10 mmol/L ammonium acetate at a 0.5 mL/min flow rate, with a column temperature of 25 °C. Detection was achieved using a single quadrupole mass spectrometer with electrospray ionization in positive mode, operated in selected ion monitoring (SIM) mode to detect creatinine (m/z 114) and creatinine- d_3 (m/z 117). The MS parameters were optimized as follows: fragmentor voltage, 90 V; dwell time, 195 msec; gain, 1; drying gas flow rate, 12 L/min; nebulizer pressure, 345 kPa (50 psig); voltage, 1500 V; and gas temperature, 350 °C. The randomized run sequence for the replicate measurements (3) of each campaign set (Campaign Set 1 and Campaign Set 2) is outlined in Appendix B, Table B2. An

IS approach and relative response factor (RRF) were used for calibration, according to the equation [6,13]:

$$\text{RRF} = \frac{(A_{\text{Creatinine,Cal}} \times M_{\text{IS,Cal}})}{(A_{\text{IS,Cal}} \times M_{\text{Creatinine,Cal}} \times P_{\text{Creatinine}})} \quad (1)$$

where $A_{\text{Creatinine,Cal}}$ represents the peak area for creatinine (unlabeled) for the calibration solution, $M_{\text{IS,Cal}}$ represents the mass of the IS in the calibration solution, $A_{\text{IS,Cal}}$ represents the peak area for the IS in the calibration solution, $M_{\text{Creatinine,Cal}}$ represents the mass of creatinine (unlabeled) in the calibration solution, and $P_{\text{Creatinine}}$ represents the purity correction factor for creatinine. The RRF was averaged from triplicate injections of each calibration solution over a narrow-bracketed mass ratio range (0.8 to 1.2 mass ratio, creatinine: d_3 -creatinine) (Appendix B, Table B3). The RRF was applied to samples to determine the mass fraction of creatinine according to the equation [6,13]:

$$\text{Mass Fraction } \left(\frac{\mu\text{g}}{\text{g}} \right) = \frac{(A_{\text{Creatinine,S}} \times M_{\text{IS,S}})}{(A_{\text{IS,S}} \times M_{\text{S}} \times \text{RRF})} \quad (2)$$

where $A_{\text{Creatinine,S}}$ represents the peak area for creatinine (unlabeled) in the sample, $M_{\text{IS,S}}$ represents the mass of the IS in the sample, $A_{\text{IS,S}}$ represents the peak area for the IS in the sample, and M_{S} represents the mass of creatinine (unlabeled) in the sample. The creatinine mass fraction ($\mu\text{g/g}$) values for the CRM samples are summarized in Appendix B, Table B4.

3. RESULTS

Urine Albumin Results. The MRM chromatographic profiles (Appendix A, Fig. A1 and A2) of the albumin MRM peptides (combined profile of unlabeled and IS) and the MRM retention time (RT) profiles (Appendix A, Fig. A3) were uniform across both CRMs. The MRM peak area ratio (PAR, unlabeled-to-IS ratio) values were consistent across the calibration solutions in Campaign Sets 1 and 2, as detailed in Appendix A, Table A4. The bracketed calibration curves showed a strong linear relationship between PAR and concentration ratio across the concentration levels for each qt-MRM transition in both campaigns (Appendix A, Fig. A4 and A5). The urine albumin mass fraction values ($X_{mg/g}$) for the CRM samples were calculated for each qt-MRM transition using the PAR values and the linear equations derived from the bracketed calibration curves. The corresponding PAR and $X_{mg/g}$ values for the qt-MRM transitions of each CRM are detailed in Appendix A, Table A5. The MRM PAR profiles for the CRM samples showed consistent results between the two campaigns (Appendix A, Fig. A6). The consistency of the PAR and mass fraction ($\mu\text{g/g}$) values between the campaigns for each CRM indicates that the results were stable and reproducible throughout the equivalence evaluation. Consensus means analysis was used to determine the consensus mass fraction value ($\bar{X}_{mg/g}$) using the qt-MRM transition $X_{mg/g}$ values. The density values for each CRM were used in the unit conversion of the consensus mass fraction ($\bar{X}_{mg/g}$) to the consensus mass concentration ($\bar{X}_{mg/L}$). The measured values (X_i) demonstrated minimal variability within and between campaigns and were in good agreement with the certified

intervals for both CRMs (Appendix A, Fig. A7), confirming the reliability and robustness of the equivalence evaluation.

Urine Creatinine Results. The calibrant peak area ratios and response factors for each campaign are detailed in Appendix B, Table B3. Prior to calculating the calibrant response factors, the masses of each calibrant stock were adjusted for purity. As expected, the response factors for both campaigns were close to unity, with mean values of 1.01210 and 1.00471 for Campaigns 1 and 2, respectively (Appendix B, Table B3). Moreover, the response factors exhibited low variability (% CV of $\leq 0.87\%$), indicating consistency in the calibrant preparation and stability, and repeatable and reproducible peak area ratios over the experimental period. Representative MRM chromatograms for the calibrants, SRM 3666 (Level I to Level IV), and HRM-3004A (Level 1 and Level 2) samples are displayed in Appendix B, Fig. B1-B3.

Figure 2 illustrates the urine creatinine experimental design for each campaign, showing that three aliquots were analyzed in triplicate, resulting in nine (9) measurements per campaign. The corresponding peak area ratio and mass fraction ($\mu\text{g/g}$) values for each sample are listed in Appendix B, Table B4. The low within- and between-Campaign variability of the measured values (X_i) for both CRMs (Appendix B, Fig. B4) demonstrates the reliability and robustness of the equivalence evaluation. Consistent with the urine albumin results, the measured creatinine values demonstrated minimal variability within and between campaigns and were in good agreement with the certified values for both CRMs (Appendix B, Fig. B4), confirming the reliability and robustness of the equivalence evaluation.

4. EXTENT-OF-EQUIVALENCE ASSESSMENT

Urine Albumin. The extent-of-equivalence data for Campaign 1 and Campaign 2 urine albumin $\bar{X}_{\text{mg/L}}$ values, including associated expanded uncertainties ($k = 2$), and summary statistics for NIST SRM 3666 and HSA HRM-3004A are summarized in Table 7. The measured values (X_i) for both CRMs were consistent with the certified values (C_i), as indicated by the overlapping 95 % confidence intervals (CIs) shown in Table 7 (Appendix A, Fig. A7).

Table 7. Urine albumin certified values, measured values, and summary statistics of CRMs for extent-of-equivalence assessment.

CRM Name ^a	Certified Values (C_i , mg/L) ^b		Measured Values (X_i , mg/L) ^c				Summary Statistics				
	Value	U_{95}	Campaign 1		Campaign 2		Mean	SD	R ^d	u_c ^e	U_{95} ^f
			Value	U_{95}	Value	U_{95}					
SRM 3666 Level I	8.28	1.12	8.36	0.95	8.02	0.93	8.19	0.24	0.47	0.53	1.06
SRM 3666 Level II	31.11	2.64	29.23	2.58	30.13	4.75	29.68	0.63	1.91	2.01	4.02
SRM 3666 Level III	112.77	10.79	111.61	6.53	104.95	6.23	108.28	4.71	3.19	5.69	11.37
SRM 3666 Level IV	360.50	31.11	341.84	16.53	359.37	13.45	350.61	12.39	7.53	14.50	29.01
HRM 3004A Level 1	40.10	2.40	38.56	3.22	39.68	3.38	39.12	0.79	1.65	1.83	3.66
HRM 3004A Level 2	226.00	11.00	217.62	9.50	222.03	9.72	219.83	3.12	4.81	5.73	11.46

^a CRMs suitable for the calibration or verification of albumin in human urine. Materials with the prefix ‘SRM’ are available from the National Institute of Standards and Technology (NIST), USA and materials with the prefix ‘HRM’ are available from Health Science Authority, Singapore.

^b Certified values and 95 % confidence level uncertainties ($k = 2$) as listed on the CRM Certificates.

^c Measured values and fully evaluated 95 % confidence level uncertainties ($k = 2$). The determinations were performed at NIST using the NIST RMP for Urine Albumin [2-4, 9]. The urine albumin content of each level of the CRMs was fully evaluated in two separately analyzed vials of the material. The two sets of vials were analyzed in separate measurement campaigns (Campaign 1 and Campaign 2).

^d Expected repeatability standard deviation of a single determination in this matrix using the NIST RMP for Urine Albumin [2-4, 9], estimated as one-half of the pooled Campaign 1 and Campaign 2 U_{95} values: $R = 0.5 \sqrt{\frac{U_{95,1}^2 + U_{95,2}^2}{2}}$.

^e Standard combined uncertainty, $u_c = \sqrt{SD^2 + R^2}$.

^f Approximate 95 % confidence expanded uncertainty, $2u_c$.

The scattergram plot illustrates the linear correlation between the certified (C_i) and measured (X_i) values for both CRMs (Fig. 3).

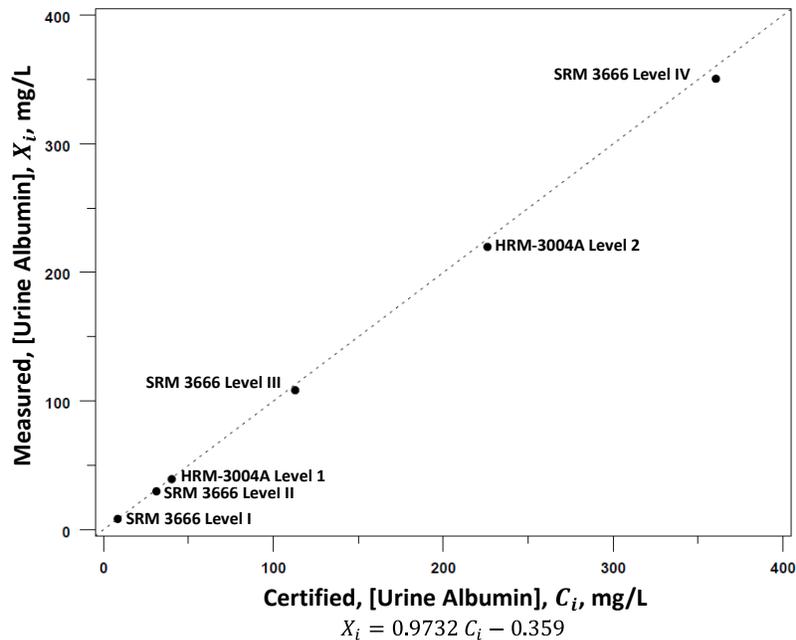


Fig. 3. Scatter plot of the extent-of-equivalence of albumin in human urine CRMs [NIST SRM 3666 (Level I to Level IV) and HSA HRM-3004A (Level 1 and Level 2)].

The horizontal axis reports the certified values, C_i and the vertical reports the measured values (consensus mass concentration values), X_i . Each level of each CRM is displayed as approximate 95 % uncertainty intervals along both axes. The dotted line denotes the linear function: $X_i = -0.359 + 0.9732 C_i$.

The linear regression analysis ($X_i = a + bC_i$) shows a strong positive correlation between the certified (C_i) and measured (X_i) values for both CRMs, with a near-zero intercept (-0.359) and a slope (0.9732) close to unity, indicating excellent agreement between the measured and certified values (Fig. 3). Figure 4 further illustrates the correlation between the certified and measured values by presenting the 95 % confidence intervals for the certified [$C_i \pm U_{95}(C_i)$] and measured [$X_i \pm U_{95}(X_i)$] values relative to the certified values ($X_i/C_i \pm U_{95}(X_i)/C_i$) and $(1 \pm U_{95}(C_i))/C_i$). The overlapping intervals for both CRMs confirm the agreement between the measured [$X_i \pm U_{95}(X_i)$] and certified [$C_i \pm U_{95}(C_i)$] intervals. This study demonstrates the equivalence of NIST SRM 3666 and HSA HRM-3004A for urine albumin measurements, supporting the use of either CRM in the calibration hierarchy for urine albumin.

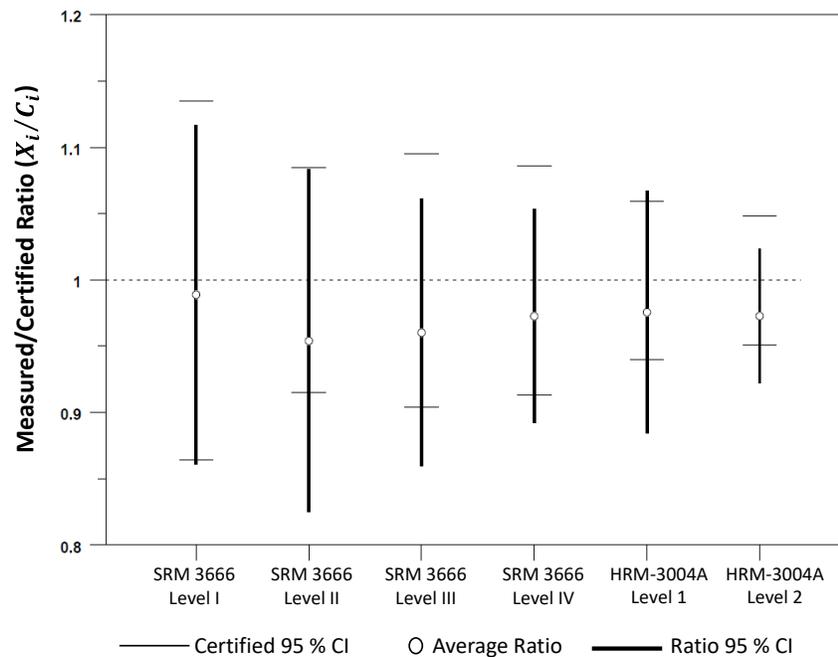


Fig. 4. Ratio plot of the extent-of-equivalence of albumin in human urine CRMs [NIST SRM 3666 (Level I to Level IV) and HSA HRM-3004A (Level 1 and Level 2)].

The horizontal axis reports the CRMs evaluated. The vertical axis reports the ratio between the average measured and certified values of each CRM (X_i/C_i). The circles denote the mean ratios; the dark vertical lines represent the approximate 95 % uncertainty interval on these means ($k = 2$). The light horizontal lines represent the certified 95 % confidence intervals (CI). The dotted line represents the expected ratio for the suite of all materials, given the observed identity between the measured (X_i) and certified (C_i) values.

Urine Creatinine. The extent-of-equivalence data for Campaign 1 and Campaign 2 urine creatinine mass concentration (mg/dL) values, including associated expanded uncertainties ($k = 2.31$), and summary statistics for NIST SRM 3666 (Level I to Level IV) and HSA HRM-3004A (Level 1 and Level 2) are summarized in Table 8. Similar to the urine albumin results, the measured values (X_i) for both CRMs were consistent with the certified values (C_i), as evidenced by the overlapping 95 % confidence intervals (CIs) presented in Table 8 (Appendix B, Fig. B4).

Table 8. Urine creatinine certified values, measured values, and summary statistics of CRMs for extent-of-equivalence assessment.

CRM Name ^a	Certified Values (C_i , mg/dL) ^b		Measured Values (X_i , mg/dL) ^c				Summary Statistics				
	Value	U_{95}	Campaign 1		Campaign 2		Mean	SD	R^d	u_c^e	U_{95}^f
			Value	U_{95}	Value	U_{95}					
SRM 3666 Level I	119.69	2.63	116.52	2.92	114.40	2.87	115.46	1.49	1.45	2.08	4.81
SRM 3666 Level II	122.65	2.73	118.49	2.99	114.51	2.85	116.50	2.82	1.46	3.17	7.33
SRM 3666 Level III	127.19	2.78	126.62	3.22	119.89	3.03	123.25	4.76	1.56	5.01	11.56
SRM 3666 Level IV	130.90	2.96	127.00	3.23	127.02	3.25	127.01	0.01	1.62	1.62	3.74
HRM 3004A Level 1	40.35	1.05	39.69	0.97	39.83	0.97	39.76	0.10	0.49	0.50	1.15
HRM 3004A Level 2	122.40	4.19	121.20	2.97	121.30	2.97	121.25	0.07	1.48	1.49	3.43

^a CRMs suitable for the calibration or verification of albumin in human urine. Materials with the prefix ‘SRM’ are available from the National Institute of Standards and Technology (NIST), USA and materials with the prefix ‘HRM’ are available from Health Science Authority, Singapore.

^b Certified values and 95 % confidence level uncertainties (NIST: $k = 2.201$ / HSA: $k = 2$) as listed on the CRM Certificates.

^c Measured values and fully evaluated 95 % confidence level uncertainties ($k = 2.31$). The determinations were performed at NIST using the NIST RMP for Creatinine [12-14]. The urine albumin content of each level of the CRMs was fully evaluated in two separately analyzed vials of the material. The two sets of vials were analyzed in separate measurement campaigns (Campaign 1 and Campaign 2).

^d Expected repeatability standard deviation of a single determination in this matrix using the NIST RMP for Creatinine [12-14], estimated as one-half of the pooled Campaign 1 and Campaign 2 U_{95} values: $R = 0.5 \sqrt{\frac{U_{95,1}^2 + U_{95,2}^2}{2}}$.

^e Standard combined uncertainty, $u_c = \sqrt{SD^2 + R^2}$.

^f Approximate 95 % confidence expanded uncertainty, $2u_c$.

The scattergram plot illustrates the linear correlation between the certified (C_i) and measured (X_i) values for both CRMs (Fig. 5).

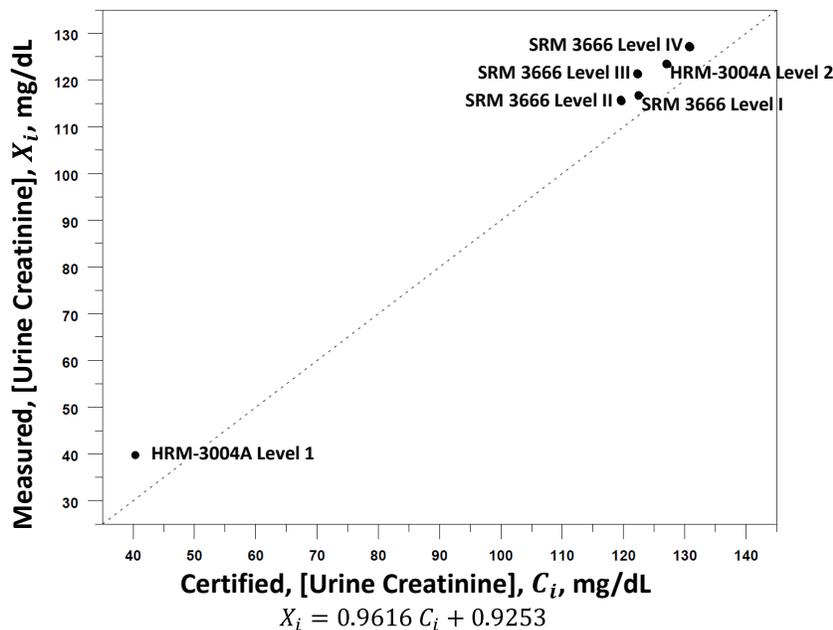


Fig. 5. Scatter plot of the extent-of-equivalence of creatinine in human urine CRMs [NIST SRM 3666 (Level I to Level IV) and HSA HRM-3004A (Level 1 and Level 2)].

The horizontal axis reports the certified values, C_i and the vertical reports the measured values (consensus mass concentration values), X_i . Each level of each CRM is displayed as approximate 95 % uncertainty intervals along both axes. The dotted line denotes the linear function: $X_i = 0.9616 C_i + 0.9253$.

The linear regression analysis (Fig. 5) reveals a positive correlation between the certified (C_i) and measured (X_i) values for both CRMs, with a slope of 0.9616 and a near-zero intercept of 0.9253, indicating agreement between the measured and certified values. Consistent with the urine albumin results, the agreement between the measured [$X_i \pm U_{95}(X_i)$] and certified [$C_i \pm U_{95}(C_i)$] intervals is further demonstrated in Fig. 6, which shows the 95 % confidence intervals for the certified [$C_i \pm U_{95}(C_i)$] and measured [$X_i \pm U_{95}(X_i)$] values relative to the certified values ($X_i/C_i \pm U_{95}(X_i)/C_i$) and $(1 \pm U_{95}(C_i))/C_i$). This study demonstrates the equivalence of NIST SRM 3666 and HSA HRM-3004A, supporting their interchangeable use in the calibration hierarchy for urine creatinine measurements.

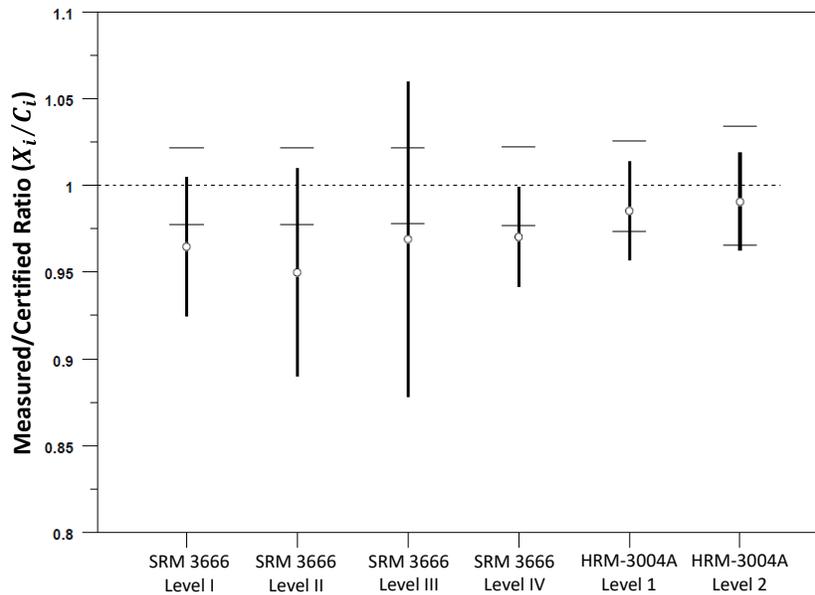


Fig. 6. Ratio plot of the extent-of-equivalence of creatinine in human urine CRMs [NIST SRM 3666 (Level I to Level IV) and HSA HRM-3004A (Level 1 and Level 2)].

The horizontal axis reports the CRMs evaluated. The vertical axis reports the ratio between the average measured and certified values of each CRM (X_i/C_i). The circles denote the mean ratios; the dark vertical lines represent the approximate 95 % uncertainty interval on these means ($k = 2.31$). The light horizontal lines represent the certified 95 % confidence intervals (CI). The dotted line represents the expected ratio for the suite of all materials given the observed identity between the measured (X_i) and certified (C_i) values.

CONCLUSION

The equivalence assessment between NIST SRM 3666 (Levels I to IV) and HSA HRM-3004A (Levels 1 and 2) for urine albumin and creatinine demonstrated overlapping measured [$X_i \pm U_{95}(X_i)$] and certified [$C_i \pm U_{95}(C_i)$] concentration intervals, indicating agreement within their assigned uncertainties at a 95 % confidence level (Tables 7 and 8; Fig. 4 and 6). This equivalence confirms that both CRMs can be used interchangeably in a calibration hierarchy for urine albumin and creatinine without introducing additional bias, thus maintaining the integrity and reliability of measurement results. The interchangeability of NIST SRM 3666 and HSA HRM-3004A has significant implications for clinical laboratory testing. It enables the comparison of clinical data

across different laboratories and methods, facilitating the development of standardized clinical guidelines. Establishing a robust calibration hierarchy for urine albumin and creatinine measurements will enhance the reliability and accuracy of clinical laboratory results for kidney disease diagnosis and monitoring, ultimately contributing to better patient outcomes and more effective healthcare management.

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APPENDIX A – URINE ALBUMIN EXTENT-OF-EQUIVALENCE MEASUREMENTS

Supplemental Data and Graphs: Extent-of-Equivalence Analysis for Urine Albumin Measurements in NIST SRM 3666 and HSA HRM-30304A

Table A1. Calibration levels and urine albumin concentration (mg/L) ranges used in the bracketed calibration approach for Campaign Set 1 and Campaign Set 2.

Campaign Set	Calibration Level	Urine Albumin Concentration Range (mg/L)
Campaign Set 1	Calibration Level 1	4.9 to 19.7
Campaign Set 1	Calibration Level 2	28.7 to 78.8
Campaign Set 1	Calibration Level 3	100.3 to 205.9
Campaign Set 1	Calibration Level 4	205.9 to 498.15
Campaign Set 2	Calibration Level 1	5.2 to 11.0
Campaign Set 2	Calibration Level 2	25.2 to 80.1
Campaign Set 2	Calibration Level 3	105.4 to 298.8
Campaign Set 2	Calibration Level 4	204.4 to 498.15

Table A2. Extent-of-equivalence samples for urine albumin measurements.

Campaign Set	Campaign (Unit)	CRM Name	Level	Aliquot#	Sample Name
Campaign Set 1	1	SRM 3666	Level I	1	N-L1.1.1
Campaign Set 1	1	SRM 3666	Level I	2	N-L1.1.2
Campaign Set 1	1	SRM 3666	Level I	3	N-L1.1.3
Campaign Set 1	1	SRM 3666	Level II	1	N-L2.1.1
Campaign Set 1	1	SRM 3666	Level II	2	N-L2.1.2
Campaign Set 1	1	SRM 3666	Level II	3	N-L2.1.3
Campaign Set 1	1	SRM 3666	Level III	1	N-L3.1.1
Campaign Set 1	1	SRM 3666	Level III	2	N-L3.1.2
Campaign Set 1	1	SRM 3666	Level III	3	N-L3.1.3
Campaign Set 1	1	SRM 3666	Level IV	1	N-L4.1.1
Campaign Set 1	1	SRM 3666	Level IV	2	N-L4.1.2
Campaign Set 1	1	SRM 3666	Level IV	3	N-L4.1.3
Campaign Set 1	1	HRM-3004A	Level 1	1	S-L1.1.1
Campaign Set 1	1	HRM-3004A	Level 1	2	S-L1.1.2
Campaign Set 1	1	HRM-3004A	Level 1	3	S-L1.1.3
Campaign Set 1	1	HRM-3004A	Level 2	1	S-L2.1.1
Campaign Set 1	1	HRM-3004A	Level 2	2	S-L2.1.2
Campaign Set 1	1	HRM-3004A	Level 2	3	S-L2.1.3
Campaign Set 2	2	SRM 3666	Level I	1	N-L1.2.1
Campaign Set 2	2	SRM 3666	Level I	2	N-L1.2.2
Campaign Set 2	2	SRM 3666	Level I	3	N-L1.2.3
Campaign Set 2	2	SRM 3666	Level II	1	N-L2.2.1
Campaign Set 2	2	SRM 3666	Level II	2	N-L2.2.2
Campaign Set 2	2	SRM 3666	Level II	3	N-L2.2.3
Campaign Set 2	2	SRM 3666	Level III	1	N-L3.2.1
Campaign Set 2	2	SRM 3666	Level III	2	N-L3.2.2
Campaign Set 2	2	SRM 3666	Level III	3	N-L3.2.3
Campaign Set 2	2	SRM 3666	Level IV	1	N-L4.2.1
Campaign Set 2	2	SRM 3666	Level IV	2	N-L4.2.2
Campaign Set 2	2	SRM 3666	Level IV	3	N-L4.2.3
Campaign Set 2	2	HRM-3004A	Level 1	1	S-L1.2.1
Campaign Set 2	2	HRM-3004A	Level 1	2	S-L1.2.2
Campaign Set 2	2	HRM-3004A	Level 1	3	S-L1.2.3
Campaign Set 2	2	HRM-3004A	Level 2	1	S-L2.2.1
Campaign Set 2	2	HRM-3004A	Level 2	2	S-L2.2.2
Campaign Set 2	2	HRM-3004A	Level 2	3	S-L2.2.3

Table A3. Randomized LC-MS/MS run sequence for Campaign Set 1 and Campaign Set 2.

Campaign Set	Sample Name	Injection Volume (µL)	Campaign Set	Sample Name	Injection Volume (µL)
Campaign Set 1	Clean1	1.0	Campaign Set 2	Clean1	1.0
Campaign Set 1	Blank1	1.0	Campaign Set 2	Blank1	1.0
Campaign Set 1	Cal 6_Set 1 Run 1	6.7	Campaign Set 2	QC-1_Set 2 Run 1	7.0
Campaign Set 1	Cal 4_Set 1 Run 1	7.9	Campaign Set 2	N-L2.2.3_Set 2 Run 1	7.1
Campaign Set 1	N-L3.1.1_Set 1 Run 1	4.1	Campaign Set 2	N-L1.2.2_Set 2 Run 1	8.9
Campaign Set 1	Cal 5_Set 1 Run 1	7.6	Campaign Set 2	S-L2.2.2_Set 2 Run 1	2.5
Campaign Set 1	Cal 9_Set 1 Run 1	4.4	Campaign Set 2	S-L1.2.1_Set 2 Run 1	6.6
Campaign Set 1	Cal 10_Set 1 Run 1	3.4	Campaign Set 2	Cal 13_Set 2 Run 1	1.6
Campaign Set 1	Cal 11_Set 1 Run 1	2.7	Campaign Set 2	Cal 12_Set 2 Run 1	2.0
Campaign Set 1	Cal 12_Set 1 Run 1	2.1	Campaign Set 2	Cal 3_Set 2 Run 1	8.3
Campaign Set 1	N-L4.1.3_Set 1 Run 1	1.8	Campaign Set 2	Cal 8_Set 2 Run 1	4.9
Campaign Set 1	QC-2_Set 1 Run 1	4.2	Campaign Set 2	Cal 6_Set 2 Run 1	6.3
Campaign Set 1	N-L1.1.3_Set 1 Run 1	9.4	Campaign Set 2	S-L2.2.1_Set 2 Run 1	2.5
Campaign Set 1	Cal 3_Set 1 Run 1	9.5	Campaign Set 2	N-L4.2.1_Set 2 Run 1	1.8
Campaign Set 1	Cal 1_Set 1 Run 1	9.9	Campaign Set 2	N-L3.2.1_Set 2 Run 1	4.0
Campaign Set 1	Cal 7_Set 1 Run 1	6.3	Campaign Set 2	N-L4.2.3_Set 2 Run 1	1.8
Campaign Set 1	S-L1.1.3_Set 1 Run 1	6.7	Campaign Set 2	Cal 10_Set 2 Run 1	3.3
Campaign Set 1	N-L2.1.1_Set 1 Run 1	7.3	Campaign Set 2	Cal 4_Set 2 Run 1	7.6
Campaign Set 1	Cal 2_Set 1 Run 1	9.8	Campaign Set 2	Cal 2_Set 2 Run 1	9.4
Campaign Set 1	N-L4.1.1_Set 1 Run 1	1.8	Campaign Set 2	N-L3.2.3_Set 2 Run 1	4.1
Campaign Set 1	S-L2.1.1_Set 1 Run 1	2.5	Campaign Set 2	N-L4.2.2_Set 2 Run 1	1.8
Campaign Set 1	N-L1.1.2_Set 1 Run 1	9.2	Campaign Set 2	S-L1.2.3_Set 2 Run 1	6.6
Campaign Set 1	N-L2.1.2_Set 1 Run 1	7.2	Campaign Set 2	Cal 11_Set 2 Run 1	2.8
Campaign Set 1	Cal 8_Set 1 Run 1	5.1	Campaign Set 2	S-L2.2.3_Set 2 Run 1	2.5
Campaign Set 1	N-L3.1.3_Set 1 Run 1	4.1	Campaign Set 2	N-L2.2.1_Set 2 Run 1	7.1
Campaign Set 1	S-L2.1.3_Set 1 Run 1	2.5	Campaign Set 2	N-L2.2.2_Set 2 Run 1	7.0
Campaign Set 1	Cal 13_Set 1 Run 1	1.6	Campaign Set 2	Cal 5_Set 2 Run 1	6.4
Campaign Set 1	N-L3.1.2_Set 1 Run 1	4.1	Campaign Set 2	Cal 9_Set 2 Run 1	4.3
Campaign Set 1	N-L1.1.1_Set 1 Run 1	9.4	Campaign Set 2	Cal 1_Set 2 Run 1	9.5
Campaign Set 1	N-L2.1.3_Set 1 Run 1	7.3	Campaign Set 2	Cal 14_Set 2 Run 1	1.4
Campaign Set 1	S-L1.1.1_Set 1 Run 1	6.7	Campaign Set 2	N-L1.2.1_Set 2 Run 1	9.1
Campaign Set 1	S-L2.1.2_Set 1 Run 1	2.5	Campaign Set 2	Cal 7_Set 2 Run 1	6.1
Campaign Set 1	Cal 14_Set 1 Run 1	2.4	Campaign Set 2	N-L1.2.3_Set 2 Run 1	9.0
Campaign Set 1	N-L4.1.2_Set 1 Run 1	1.8	Campaign Set 2	S-L1.2.2_Set 2 Run 1	6.5
Campaign Set 1	S-L1.1.2_Set 1 Run 1	6.6	Campaign Set 2	N-L3.2.2_Set 2 Run 1	4.0
Campaign Set 1	Clean2	1.0	Campaign Set 2	Clean2	1.0
Campaign Set 1	Blank2	1.0	Campaign Set 2	Blank2	1.0
Campaign Set 1	Cal 3_Set 1 Run 2	9.5	Campaign Set 2	S-L1.2.1_Set 2 Run 2	6.6
Campaign Set 1	Cal 13_Set 1 Run 2	1.6	Campaign Set 2	Cal 2_Set 2 Run 2	9.4
Campaign Set 1	N-L2.1.3_Set 1 Run 2	7.3	Campaign Set 2	S-L2.2.1_Set 2 Run 2	2.5
Campaign Set 1	N-L2.1.2_Set 1 Run 2	7.2	Campaign Set 2	S-L2.2.2_Set 2 Run 2	2.5
Campaign Set 1	Cal 1_Set 1 Run 2	9.9	Campaign Set 2	Cal 7_Set 2 Run 2	6.1
Campaign Set 1	Cal 5_Set 1 Run 2	7.6	Campaign Set 2	N-L2.2.2_Set 2 Run 2	7.0
Campaign Set 1	Cal 10_Set 1 Run 2	3.4	Campaign Set 2	S-L1.2.2_Set 2 Run 2	6.5
Campaign Set 1	Cal 2_Set 1 Run 2	9.8	Campaign Set 2	Cal 13_Set 2 Run 2	1.6
Campaign Set 1	Cal 8_Set 1 Run 2	5.1	Campaign Set 2	N-L3.2.3_Set 2 Run 2	4.1
Campaign Set 1	S-L1.1.1_Set 1 Run 2	6.7	Campaign Set 2	N-L4.2.3_Set 2 Run 2	1.8
Campaign Set 1	Cal 6_Set 1 Run 2	6.7	Campaign Set 2	Cal 8_Set 2 Run 2	4.9
Campaign Set 1	N-L1.1.1_Set 1 Run 2	9.4	Campaign Set 2	N-L1.2.3_Set 2 Run 2	9.0
Campaign Set 1	QC-2_Set 1 Run 2	4.2	Campaign Set 2	Cal 5_Set 2 Run 2	6.4
Campaign Set 1	N-L2.1.1_Set 1 Run 2	7.3	Campaign Set 2	N-L1.2.2_Set 2 Run 2	8.9
Campaign Set 1	S-L2.1.2_Set 1 Run 2	2.5	Campaign Set 2	Cal 3_Set 2 Run 2	8.3
Campaign Set 1	Cal 9_Set 1 Run 2	4.4	Campaign Set 2	N-L3.2.1_Set 2 Run 2	4.0
Campaign Set 1	Cal 4_Set 1 Run 2	7.9	Campaign Set 2	Cal 4_Set 2 Run 2	7.6
Campaign Set 1	N-L4.1.3_Set 1 Run 2	1.8	Campaign Set 2	QC-1_Set 2 Run 2	7.0
Campaign Set 1	N-L1.1.2_Set 1 Run 2	9.2	Campaign Set 2	N-L2.2.3_Set 2 Run 2	7.1
Campaign Set 1	N-L1.1.3_Set 1 Run 2	9.4	Campaign Set 2	Cal 11_Set 2 Run 2	2.8
Campaign Set 1	Cal 7_Set 1 Run 2	6.3	Campaign Set 2	N-L3.2.2_Set 2 Run 2	4.0
Campaign Set 1	Cal 12_Set 1 Run 2	2.1	Campaign Set 2	S-L1.2.3_Set 2 Run 2	6.6
Campaign Set 1	N-L4.1.1_Set 1 Run 2	1.8	Campaign Set 2	S-L2.2.3_Set 2 Run 2	2.5

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Campaign Set 1	N-L4.1.2_Set 1 Run 2	1.8	Campaign Set 2	Cal 12_Set 2 Run 2	2.0
Campaign Set 1	N-L3.1.1_Set 1 Run 2	4.1	Campaign Set 2	N-L2.2.1_Set 2 Run 2	7.1
Campaign Set 1	N-L3.1.3_Set 1 Run 2	4.1	Campaign Set 2	Cal 9_Set 2 Run 2	4.3
Campaign Set 1	N-L3.1.2_Set 1 Run 2	4.1	Campaign Set 2	N-L1.2.1_Set 2 Run 2	9.1
Campaign Set 1	S-L1.1.3_Set 1 Run 2	6.7	Campaign Set 2	Cal 14_Set 2 Run 2	1.4
Campaign Set 1	S-L2.1.1_Set 1 Run 2	2.5	Campaign Set 2	N-L4.2.1_Set 2 Run 2	1.8
Campaign Set 1	S-L1.1.2_Set 1 Run 2	6.6	Campaign Set 2	Cal 6_Set 2 Run 2	6.3
Campaign Set 1	S-L2.1.3_Set 1 Run 2	2.5	Campaign Set 2	Cal 1_Set 2 Run 2	9.5
Campaign Set 1	Cal 14_Set 1 Run 2	2.4	Campaign Set 2	Cal 10_Set 2 Run 2	3.3
Campaign Set 1	Cal 11_Set 1 Run 2	2.7	Campaign Set 2	N-L4.2.2_Set 2 Run 2	1.8
Campaign Set 1	Clean3	1.0	Campaign Set 2	Clean3	1.0
Campaign Set 1	Blank3	1.0	Campaign Set 2	Blank3	1.0
Campaign Set 1	Cal 13_Set 1 Run 3	1.6	Campaign Set 2	S-L1.2.1_Set 2 Run 3	6.6
Campaign Set 1	Cal 11_Set 1 Run 3	2.7	Campaign Set 2	Cal 6_Set 2 Run 3	6.3
Campaign Set 1	S-L2.1.1_Set 1 Run 3	2.5	Campaign Set 2	N-L4.2.2_Set 2 Run 3	1.8
Campaign Set 1	S-L2.1.2_Set 1 Run 3	2.5	Campaign Set 2	Cal 10_Set 2 Run 3	3.3
Campaign Set 1	Cal 6_Set 1 Run 3	6.7	Campaign Set 2	N-L2.2.3_Set 2 Run 3	7.1
Campaign Set 1	Cal 12_Set 1 Run 3	2.1	Campaign Set 2	Cal 9_Set 2 Run 3	4.3
Campaign Set 1	Cal 9_Set 1 Run 3	4.4	Campaign Set 2	S-L1.2.3_Set 2 Run 3	6.6
Campaign Set 1	N-L3.1.1_Set 1 Run 3	4.1	Campaign Set 2	S-L2.2.3_Set 2 Run 3	2.5
Campaign Set 1	N-L4.1.3_Set 1 Run 3	1.8	Campaign Set 2	Cal 11_Set 2 Run 3	2.8
Campaign Set 1	N-L4.1.2_Set 1 Run 3	1.8	Campaign Set 2	Cal 14_Set 2 Run 3	1.4
Campaign Set 1	Cal 14_Set 1 Run 3	2.4	Campaign Set 2	Cal 1_Set 2 Run 3	9.5
Campaign Set 1	S-L1.1.3_Set 1 Run 3	6.7	Campaign Set 2	S-L2.2.2_Set 2 Run 3	2.5
Campaign Set 1	N-L2.1.2_Set 1 Run 3	7.2	Campaign Set 2	Cal 2_Set 2 Run 3	9.4
Campaign Set 1	N-L1.1.1_Set 1 Run 3	9.4	Campaign Set 2	Cal 5_Set 2 Run 3	6.4
Campaign Set 1	N-L3.1.2_Set 1 Run 3	4.1	Campaign Set 2	N-L4.2.3_Set 2 Run 3	1.8
Campaign Set 1	Cal 7_Set 1 Run 3	6.3	Campaign Set 2	Cal 13_Set 2 Run 3	1.6
Campaign Set 1	N-L4.1.1_Set 1 Run 3	1.8	Campaign Set 2	N-L2.2.1_Set 2 Run 3	7.1
Campaign Set 1	N-L3.1.3_Set 1 Run 3	4.1	Campaign Set 2	N-L4.2.1_Set 2 Run 3	1.8
Campaign Set 1	N-L2.1.1_Set 1 Run 3	7.3	Campaign Set 2	N-L3.2.1_Set 2 Run 3	4.0
Campaign Set 1	Cal 8_Set 1 Run 3	5.1	Campaign Set 2	S-L2.2.1_Set 2 Run 3	2.5
Campaign Set 1	Cal 4_Set 1 Run 3	7.9	Campaign Set 2	N-L1.2.2_Set 2 Run 3	8.9
Campaign Set 1	N-L1.1.2_Set 1 Run 3	9.2	Campaign Set 2	Cal 7_Set 2 Run 3	6.1
Campaign Set 1	Cal 3_Set 1 Run 3	9.5	Campaign Set 2	N-L3.2.3_Set 2 Run 3	4.1
Campaign Set 1	S-L2.1.3_Set 1 Run 3	2.5	Campaign Set 2	N-L2.2.2_Set 2 Run 3	7.0
Campaign Set 1	Cal 10_Set 1 Run 3	3.4	Campaign Set 2	Cal 4_Set 2 Run 3	7.6
Campaign Set 1	Cal 5_Set 1 Run 3	7.6	Campaign Set 2	QC-1_Set 2 Run 3	7.0
Campaign Set 1	S-L1.1.2_Set 1 Run 3	6.6	Campaign Set 2	Cal 3_Set 2 Run 3	8.3
Campaign Set 1	S-L1.1.1_Set 1 Run 3	6.7	Campaign Set 2	Cal 8_Set 2 Run 3	4.9
Campaign Set 1	Cal 2_Set 1 Run 3	9.8	Campaign Set 2	N-L3.2.2_Set 2 Run 3	4.0
Campaign Set 1	N-L1.1.3_Set 1 Run 3	9.4	Campaign Set 2	S-L1.2.2_Set 2 Run 3	6.5
Campaign Set 1	QC-2_Set 1 Run 3	4.2	Campaign Set 2	Cal 12_Set 2 Run 3	2.0
Campaign Set 1	Cal 1_Set 1 Run 3	9.9	Campaign Set 2	N-L1.2.1_Set 2 Run 3	9.1
Campaign Set 1	N-L2.1.3_Set 1 Run 3	7.3	Campaign Set 2	N-L1.2.3_Set 2 Run 3	9.0
Campaign Set 1	Clean4	1.0	Campaign Set 2	Clean4	1.0
Campaign Set 1	Blank4	1.0	Campaign Set 2	Blank4	1.0
Campaign Set 1	N-L1.1.1_Set 1 Run 4	9.4	Campaign Set 2	Cal 14_Set 2 Run 4	1.4
Campaign Set 1	Cal 4_Set 1 Run 4	7.9	Campaign Set 2	N-L2.2.3_Set 2 Run 4	7.1
Campaign Set 1	N-L4.1.2_Set 1 Run 4	1.8	Campaign Set 2	Cal 4_Set 2 Run 4	7.6
Campaign Set 1	N-L3.1.2_Set 1 Run 4	4.1	Campaign Set 2	N-L1.2.1_Set 2 Run 4	9.1
Campaign Set 1	Cal 14_Set 1 Run 4	2.4	Campaign Set 2	Cal 12_Set 2 Run 4	2.0
Campaign Set 1	S-L2.1.1_Set 1 Run 4	2.5	Campaign Set 2	N-L3.2.3_Set 2 Run 4	4.1
Campaign Set 1	Cal 12_Set 1 Run 4	2.1	Campaign Set 2	N-L4.2.1_Set 2 Run 4	1.8
Campaign Set 1	N-L1.1.3_Set 1 Run 4	9.4	Campaign Set 2	S-L2.2.3_Set 2 Run 4	2.5
Campaign Set 1	Cal 2_Set 1 Run 4	9.8	Campaign Set 2	N-L3.2.1_Set 2 Run 4	4.0
Campaign Set 1	Cal 8_Set 1 Run 4	5.1	Campaign Set 2	N-L4.2.3_Set 2 Run 4	1.8
Campaign Set 1	S-L2.1.3_Set 1 Run 4	2.5	Campaign Set 2	Cal 8_Set 2 Run 4	4.9
Campaign Set 1	S-L2.1.2_Set 1 Run 4	2.5	Campaign Set 2	Cal 3_Set 2 Run 4	8.3
Campaign Set 1	N-L1.1.2_Set 1 Run 4	9.2	Campaign Set 2	N-L4.2.2_Set 2 Run 4	1.8
Campaign Set 1	N-L3.1.3_Set 1 Run 4	4.1	Campaign Set 2	Cal 7_Set 2 Run 4	6.1
Campaign Set 1	N-L2.1.1_Set 1 Run 4	7.3	Campaign Set 2	N-L2.2.2_Set 2 Run 4	7.0
Campaign Set 1	S-L1.1.3_Set 1 Run 4	6.7	Campaign Set 2	Cal 9_Set 2 Run 4	4.3
Campaign Set 1	Cal 1_Set 1 Run 4	9.9	Campaign Set 2	Cal 11_Set 2 Run 4	2.8

Campaign Set 1	Cal 7_Set 1 Run 4	6.3	Campaign Set 2	QC-1_Set 2 Run 4	7.0
Campaign Set 1	S-L1.1.1_Set 1 Run 4	6.7	Campaign Set 2	Cal 10_Set 2 Run 4	3.3
Campaign Set 1	S-L1.1.2_Set 1 Run 4	6.6	Campaign Set 2	N-L2.2.1_Set 2 Run 4	7.1
Campaign Set 1	Cal 3_Set 1 Run 4	9.5	Campaign Set 2	N-L1.2.2_Set 2 Run 4	8.9
Campaign Set 1	N-L4.1.1_Set 1 Run 4	1.8	Campaign Set 2	N-L3.2.2_Set 2 Run 4	4.0
Campaign Set 1	N-L4.1.3_Set 1 Run 4	1.8	Campaign Set 2	S-L2.2.1_Set 2 Run 4	2.5
Campaign Set 1	QC-2_Set 1 Run 4	4.2	Campaign Set 2	Cal 1_Set 2 Run 4	9.5
Campaign Set 1	N-L2.1.3_Set 1 Run 4	7.3	Campaign Set 2	Cal 5_Set 2 Run 4	6.4
Campaign Set 1	Cal 6_Set 1 Run 4	6.7	Campaign Set 2	Cal 13_Set 2 Run 4	1.6
Campaign Set 1	Cal 13_Set 1 Run 4	1.6	Campaign Set 2	S-L1.2.1_Set 2 Run 4	6.6
Campaign Set 1	Cal 10_Set 1 Run 4	3.4	Campaign Set 2	N-L1.2.3_Set 2 Run 4	9.0
Campaign Set 1	Cal 9_Set 1 Run 4	4.4	Campaign Set 2	S-L1.2.3_Set 2 Run 4	6.6
Campaign Set 1	Cal 11_Set 1 Run 4	2.7	Campaign Set 2	Cal 2_Set 2 Run 4	9.4
Campaign Set 1	N-L3.1.1_Set 1 Run 4	4.1	Campaign Set 2	S-L1.2.2_Set 2 Run 4	6.5
Campaign Set 1	N-L2.1.2_Set 1 Run 4	7.2	Campaign Set 2	S-L2.2.2_Set 2 Run 4	2.5
Campaign Set 1	Cal 5_Set 1 Run 4	7.6	Campaign Set 2	Cal 6_Set 2 Run 4	6.3
Campaign Set 1	Clean5	1.0	Campaign Set 2	Clean5	1.0
Campaign Set 1	Blank5	1.0	Campaign Set 2	Blank5	1.0

Table A4. Urine albumin concentration ratio and PAR values for the calibration solutions for Campaign Set 1 and Campaign Set 2.

Campaign Set	Calibration Level	Sample Name	Sample Type	Concentration Ratio	Peak Area Ratio (PAR) (n=4)					Calibration Summary Statistics for PAR (n=20)		
					HSA 11.1	HSA 12.4	HSA 13.3	HSA 31.1	HSA 5.2	Mean PAR	Stdev	%CV
Campaign Set 1	Calibration Level 1	Calibrant 1	Cal 1	0.07	0.05	0.07	0.06	0.07	0.07	0.06	0.01	14.8
Campaign Set 1	Calibration Level 1	Calibrant 2	Cal 2	0.11	0.07	0.10	0.09	0.10	0.10	0.09	0.01	13.9
Campaign Set 1	Calibration Level 1	Calibrant 3	Cal 3	0.15	0.11	0.16	0.13	0.15	0.15	0.14	0.02	13.4
Campaign Set 1	Calibration Level 1	Calibrant 4	Cal 4	0.28	0.21	0.28	0.23	0.27	0.25	0.25	0.03	11.6
Campaign Set 1	Calibration Level 2	Calibrant 5	Cal 5	0.39	0.28	0.37	0.31	0.36	0.34	0.33	0.03	10.5
Campaign Set 1	Calibration Level 2	Calibrant 6	Cal 6	0.50	0.37	0.47	0.39	0.45	0.42	0.42	0.04	9.6
Campaign Set 1	Calibration Level 2	Calibrant 7	Cal 7	0.64	0.45	0.56	0.48	0.55	0.51	0.51	0.05	9.3
Campaign Set 1	Calibration Level 2	Calibrant 8	Cal 8	1.06	0.76	0.89	0.78	0.87	0.82	0.82	0.05	6.5
Campaign Set 1	Calibration Level 3	Calibrant 9	Cal 9	1.34	0.95	1.08	0.96	1.05	0.99	1.01	0.06	5.5
Campaign Set 1	Calibration Level 3	Calibrant 10	QC-2	1.52	1.13	1.20	1.08	1.18	1.10	1.14	0.05	4.5
Campaign Set 1	Calibration Level 3	Calibrant 11	Cal 10	1.96	1.36	1.48	1.34	1.45	1.37	1.40	0.06	4.6
Campaign Set 1	Calibration Level 3 and Level 4	Calibrant 12	Cal 11	2.87	1.95	2.02	1.89	2.01	1.89	1.95	0.06	3.2
Campaign Set 1	Calibration Level 3 and Level 4	Calibrant 13	Cal 12	3.98	2.68	2.70	2.58	2.70	2.55	2.64	0.07	2.7
Campaign Set 1	Calibration Level 4	Calibrant 14	Cal 13	5.26	3.60	3.50	3.37	3.49	3.28	3.45	0.12	3.6
Campaign Set 1	Calibration Level 4	Calibrant 15	Cal 14	6.50	4.60	4.30	4.35	4.39	4.08	4.34	0.19	4.3
Campaign Set 2	Calibration Level 1	Calibrant 1	Cal 1	0.07	0.05	0.08	0.06	0.08	0.07	0.07	0.01	15.2
Campaign Set 2	Calibration Level 1	Calibrant 2	Cal 2	0.10	0.08	0.12	0.09	0.11	0.11	0.10	0.02	14.8
Campaign Set 2	Calibration Level 1	Calibrant 3	Cal 3	0.15	0.12	0.16	0.13	0.15	0.15	0.14	0.02	12.9
Campaign Set 2	Calibration Level 2	Calibrant 5	QC-1	0.31	0.26	0.34	0.28	0.32	0.31	0.30	0.03	11.5
Campaign Set 2	Calibration Level 2	Calibrant 6	Cal 5	0.38	0.30	0.40	0.32	0.37	0.36	0.35	0.04	11.5
Campaign Set 2	Calibration Level 2	Calibrant 7	Cal 6	0.50	0.39	0.50	0.42	0.48	0.46	0.45	0.05	10.0
Campaign Set 2	Calibration Level 2	Calibrant 8	Cal 7	0.65	0.51	0.63	0.52	0.60	0.57	0.56	0.05	8.9
Campaign Set 2	Calibration Level 3	Calibrant 9	Cal 8	1.04	0.80	0.93	0.82	0.91	0.85	0.86	0.06	6.4
Campaign Set 2	Calibration Level 3	Calibrant 10	Cal 9	1.37	1.13	1.25	1.12	1.22	1.15	1.18	0.06	4.9
Campaign Set 2	Calibration Level 3	Calibrant 11	Cal 10	1.85	1.36	1.46	1.34	1.45	1.35	1.39	0.06	4.2
Campaign Set 2	Calibration Level 3 and Level 4	Calibrant 12	Cal 11	2.66	1.88	1.91	1.80	1.91	1.78	1.86	0.06	3.4
Campaign Set 2	Calibration Level 3 and Level 4	Calibrant 13	Cal 12	4.02	2.76	2.72	2.61	2.73	2.55	2.67	0.09	3.3
Campaign Set 2	Calibration Level 4	Calibrant 14	Cal 13	5.31	3.73	3.62	3.55	3.64	3.42	3.59	0.11	3.2
Campaign Set 2	Calibration Level 4	Calibrant 15	Cal 14	6.50	4.60	4.30	4.35	4.39	4.08	4.34	0.19	4.3

Table A5. Peak area ratio (PAR) and mass fraction (mg/g) values for the qt-MRM transitions for CRM samples for Campaign Set 1 and Campaign Set 2.

Campaign Set	Sample Type	CRM Name	Level	Aliquot #	Technical Replicate	Peak Area Ratio (PAR)					Urine Albumin Mass Fraction (mg/g)				
						HSA	HSA	HSA	HSA	HSA	HSA	HSA	HSA	HSA	HSA
						11.1	12.4	13.3	31.1	5.2	11.1	12.4	13.3	31.1	5.2
Campaign Set 1	N-L1.1.1	SRM 3666	Level I	1	Run 1	0.08	0.12	0.09	0.11	0.10	0.01	0.01	0.01	0.01	0.01
Campaign Set 1	N-L1.1.1	SRM 3666	Level I	1	Run 2	0.08	0.12	0.09	0.11	0.10	0.01	0.01	0.01	0.01	0.01
Campaign Set 1	N-L1.1.1	SRM 3666	Level I	1	Run 3	0.08	0.12	0.09	0.11	0.10	0.01	0.01	0.01	0.01	0.01
Campaign Set 1	N-L1.1.1	SRM 3666	Level I	1	Run 4	0.08	0.12	0.09	0.11	0.11	0.01	0.01	0.01	0.01	0.01
Campaign Set 1	N-L1.1.2	SRM 3666	Level I	2	Run 1	0.08	0.12	0.10	0.11	0.10	0.01	0.01	0.01	0.01	0.01
Campaign Set 1	N-L1.1.2	SRM 3666	Level I	2	Run 2	0.08	0.12	0.10	0.11	0.11	0.01	0.01	0.01	0.01	0.01
Campaign Set 1	N-L1.1.2	SRM 3666	Level I	2	Run 3	0.08	0.12	0.10	0.11	0.11	0.01	0.01	0.01	0.01	0.01
Campaign Set 1	N-L1.1.2	SRM 3666	Level I	2	Run 4	0.08	0.12	0.09	0.11	0.11	0.01	0.01	0.01	0.01	0.01
Campaign Set 1	N-L1.1.3	SRM 3666	Level I	3	Run 1	0.08	0.11	0.09	0.11	0.10	0.01	0.01	0.01	0.01	0.01
Campaign Set 1	N-L1.1.3	SRM 3666	Level I	3	Run 2	0.08	0.11	0.09	0.11	0.10	0.01	0.01	0.01	0.01	0.01
Campaign Set 1	N-L1.1.3	SRM 3666	Level I	3	Run 3	0.08	0.11	0.09	0.11	0.10	0.01	0.01	0.01	0.01	0.01
Campaign Set 1	N-L1.1.3	SRM 3666	Level I	3	Run 4	0.08	0.11	0.09	0.11	0.10	0.01	0.01	0.01	0.01	0.01
Campaign Set 1	N-L2.1.1	SRM 3666	Level II	1	Run 1	0.30	0.39	0.32	0.37	0.35	0.03	0.03	0.03	0.03	0.03
Campaign Set 1	N-L2.1.1	SRM 3666	Level II	1	Run 2	0.28	0.38	0.31	0.37	0.34	0.03	0.03	0.03	0.03	0.03
Campaign Set 1	N-L2.1.1	SRM 3666	Level II	1	Run 3	0.29	0.38	0.31	0.37	0.34	0.03	0.03	0.03	0.03	0.03
Campaign Set 1	N-L2.1.1	SRM 3666	Level II	1	Run 4	0.29	0.38	0.31	0.37	0.34	0.03	0.03	0.03	0.03	0.03
Campaign Set 1	N-L2.1.2	SRM 3666	Level II	2	Run 1	0.27	0.36	0.31	0.35	0.33	0.03	0.03	0.03	0.03	0.03
Campaign Set 1	N-L2.1.2	SRM 3666	Level II	2	Run 2	0.29	0.37	0.31	0.36	0.33	0.03	0.03	0.03	0.03	0.03
Campaign Set 1	N-L2.1.2	SRM 3666	Level II	2	Run 3	0.28	0.36	0.31	0.36	0.33	0.03	0.03	0.03	0.03	0.03
Campaign Set 1	N-L2.1.2	SRM 3666	Level II	2	Run 4	0.29	0.36	0.30	0.35	0.33	0.03	0.03	0.03	0.03	0.03
Campaign Set 1	N-L2.1.3	SRM 3666	Level II	3	Run 1	0.28	0.37	0.31	0.36	0.34	0.03	0.03	0.03	0.03	0.03
Campaign Set 1	N-L2.1.3	SRM 3666	Level II	3	Run 2	0.28	0.37	0.31	0.35	0.34	0.03	0.03	0.03	0.03	0.03
Campaign Set 1	N-L2.1.3	SRM 3666	Level II	3	Run 3	0.28	0.37	0.31	0.35	0.34	0.03	0.03	0.03	0.03	0.03
Campaign Set 1	N-L2.1.3	SRM 3666	Level II	3	Run 4	0.28	0.37	0.31	0.36	0.33	0.03	0.03	0.03	0.03	0.03
Campaign Set 1	N-L3.1.1	SRM 3666	Level III	1	Run 1	1.11	1.09	1.07	1.19	1.09	0.11	0.10	0.11	0.11	0.11
Campaign Set 1	N-L3.1.1	SRM 3666	Level III	1	Run 2	1.08	1.09	1.07	1.17	1.10	0.11	0.10	0.11	0.11	0.11
Campaign Set 1	N-L3.1.1	SRM 3666	Level III	1	Run 3	1.05	1.09	1.06	1.18	1.09	0.11	0.10	0.11	0.11	0.11
Campaign Set 1	N-L3.1.1	SRM 3666	Level III	1	Run 4	1.07	1.08	1.08	1.14	1.10	0.11	0.10	0.11	0.11	0.11
Campaign Set 1	N-L3.1.2	SRM 3666	Level III	2	Run 1	1.13	1.11	1.08	1.19	1.11	0.12	0.10	0.11	0.11	0.11
Campaign Set 1	N-L3.1.2	SRM 3666	Level III	2	Run 2	1.10	1.11	1.09	1.17	1.10	0.11	0.10	0.11	0.11	0.11
Campaign Set 1	N-L3.1.2	SRM 3666	Level III	2	Run 3	1.09	1.08	1.09	1.18	1.11	0.11	0.10	0.11	0.11	0.11
Campaign Set 1	N-L3.1.2	SRM 3666	Level III	2	Run 4	1.09	1.07	1.09	1.19	1.12	0.11	0.10	0.11	0.11	0.11
Campaign Set 1	N-L3.1.3	SRM 3666	Level III	3	Run 1	1.13	1.10	1.08	1.19	1.10	0.12	0.10	0.11	0.11	0.11
Campaign Set 1	N-L3.1.3	SRM 3666	Level III	3	Run 2	1.08	1.09	1.08	1.18	1.11	0.11	0.10	0.11	0.11	0.11
Campaign Set 1	N-L3.1.3	SRM 3666	Level III	3	Run 3	1.07	1.10	1.08	1.18	1.11	0.11	0.10	0.11	0.11	0.11
Campaign Set 1	N-L3.1.3	SRM 3666	Level III	3	Run 4	1.06	1.07	1.07	1.15	1.11	0.11	0.10	0.11	0.11	0.11
Campaign Set 1	N-L4.1.1	SRM 3666	Level IV	1	Run 1	3.17	3.04	2.91	3.13	2.88	0.34	0.33	0.33	0.34	0.34
Campaign Set 1	N-L4.1.1	SRM 3666	Level IV	1	Run 2	2.96	3.06	2.90	3.10	2.90	0.32	0.34	0.33	0.34	0.34
Campaign Set 1	N-L4.1.1	SRM 3666	Level IV	1	Run 3	3.01	2.99	2.93	3.15	2.87	0.32	0.33	0.33	0.35	0.34
Campaign Set 1	N-L4.1.1	SRM 3666	Level IV	1	Run 4	3.03	3.04	2.93	3.12	2.89	0.33	0.33	0.33	0.34	0.34

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Campaign Set 1	N-L4.1.2	SRM 3666	Level IV	2	Run 1	3.05	3.08	2.90	3.14	2.87	0.33	0.34	0.33	0.35	0.34
Campaign Set 1	N-L4.1.2	SRM 3666	Level IV	2	Run 2	3.11	3.01	2.97	3.13	2.88	0.34	0.33	0.34	0.34	0.34
Campaign Set 1	N-L4.1.2	SRM 3666	Level IV	2	Run 3	3.02	3.05	2.95	3.11	2.88	0.33	0.34	0.34	0.34	0.34
Campaign Set 1	N-L4.1.2	SRM 3666	Level IV	2	Run 4	3.04	3.06	2.94	3.14	2.88	0.33	0.34	0.34	0.35	0.34
Campaign Set 1	N-L4.1.3	SRM 3666	Level IV	3	Run 1	3.20	3.13	2.98	3.12	2.91	0.34	0.34	0.33	0.34	0.34
Campaign Set 1	N-L4.1.3	SRM 3666	Level IV	3	Run 2	3.06	3.08	2.98	3.17	2.88	0.33	0.34	0.34	0.35	0.33
Campaign Set 1	N-L4.1.3	SRM 3666	Level IV	3	Run 3	3.17	3.08	2.98	3.14	2.92	0.34	0.34	0.34	0.34	0.34
Campaign Set 1	N-L4.1.3	SRM 3666	Level IV	3	Run 4	3.03	3.14	2.99	3.17	2.89	0.32	0.34	0.34	0.35	0.33
Campaign Set 1	S-L1.1.1	HRM 3004A-01	Level 1	1	Run 1	0.38	0.48	0.41	0.46	0.44	0.04	0.04	0.04	0.04	0.04
Campaign Set 1	S-L1.1.1	HRM 3004A-01	Level 1	1	Run 2	0.38	0.48	0.40	0.46	0.43	0.04	0.04	0.04	0.04	0.04
Campaign Set 1	S-L1.1.1	HRM 3004A-01	Level 1	1	Run 3	0.37	0.48	0.41	0.46	0.43	0.04	0.04	0.04	0.04	0.04
Campaign Set 1	S-L1.1.1	HRM 3004A-01	Level 1	1	Run 4	0.37	0.47	0.41	0.46	0.43	0.04	0.04	0.04	0.04	0.04
Campaign Set 1	S-L1.1.2	HRM 3004A-01	Level 1	2	Run 1	0.37	0.46	0.39	0.45	0.42	0.04	0.04	0.04	0.04	0.04
Campaign Set 1	S-L1.1.2	HRM 3004A-01	Level 1	2	Run 2	0.36	0.46	0.39	0.44	0.42	0.04	0.04	0.04	0.04	0.04
Campaign Set 1	S-L1.1.2	HRM 3004A-01	Level 1	2	Run 3	0.35	0.46	0.38	0.44	0.42	0.04	0.04	0.04	0.04	0.04
Campaign Set 1	S-L1.1.2	HRM 3004A-01	Level 1	2	Run 4	0.36	0.46	0.39	0.44	0.41	0.04	0.04	0.04	0.04	0.04
Campaign Set 1	S-L1.1.3	HRM 3004A-01	Level 1	3	Run 1	0.37	0.46	0.39	0.45	0.42	0.04	0.04	0.04	0.04	0.04
Campaign Set 1	S-L1.1.3	HRM 3004A-01	Level 1	3	Run 2	0.37	0.46	0.39	0.45	0.42	0.04	0.04	0.04	0.04	0.04
Campaign Set 1	S-L1.1.3	HRM 3004A-01	Level 1	3	Run 3	0.35	0.46	0.39	0.45	0.42	0.04	0.04	0.04	0.04	0.04
Campaign Set 1	S-L1.1.3	HRM 3004A-01	Level 1	3	Run 4	0.36	0.46	0.39	0.45	0.42	0.04	0.04	0.04	0.04	0.04
Campaign Set 1	S-L2.1.1	HRM 3004A-02	Level 2	1	Run 1	1.93	2.02	1.90	2.01	1.86	0.22	0.22	0.22	0.22	0.21
Campaign Set 1	S-L2.1.1	HRM 3004A-02	Level 2	1	Run 2	2.01	2.01	1.89	1.99	1.89	0.22	0.21	0.22	0.21	0.21
Campaign Set 1	S-L2.1.1	HRM 3004A-02	Level 2	1	Run 3	1.96	2.01	1.89	2.01	1.88	0.22	0.21	0.22	0.22	0.21
Campaign Set 1	S-L2.1.1	HRM 3004A-02	Level 2	1	Run 4	1.93	1.99	1.89	2.02	1.92	0.22	0.21	0.22	0.22	0.22
Campaign Set 1	S-L2.1.2	HRM 3004A-02	Level 2	2	Run 1	1.97	2.08	1.89	2.05	1.91	0.22	0.22	0.22	0.22	0.21
Campaign Set 1	S-L2.1.2	HRM 3004A-02	Level 2	2	Run 2	1.96	2.04	1.92	2.01	1.93	0.22	0.21	0.22	0.21	0.22
Campaign Set 1	S-L2.1.2	HRM 3004A-02	Level 2	2	Run 3	1.95	2.03	1.90	2.01	1.91	0.21	0.21	0.22	0.21	0.21
Campaign Set 1	S-L2.1.2	HRM 3004A-02	Level 2	2	Run 4	1.89	2.03	1.89	2.02	1.92	0.21	0.21	0.22	0.21	0.22
Campaign Set 1	S-L2.1.3	HRM 3004A-02	Level 2	3	Run 1	1.99	2.01	1.87	2.01	1.88	0.22	0.21	0.22	0.22	0.21
Campaign Set 1	S-L2.1.3	HRM 3004A-02	Level 2	3	Run 2	1.93	1.99	1.87	2.01	1.87	0.22	0.21	0.22	0.22	0.21
Campaign Set 1	S-L2.1.3	HRM 3004A-02	Level 2	3	Run 3	1.90	1.99	1.87	1.99	1.88	0.21	0.21	0.22	0.21	0.21
Campaign Set 1	S-L2.1.3	HRM 3004A-02	Level 2	3	Run 4	1.96	2.00	1.87	2.01	1.87	0.22	0.21	0.22	0.22	0.21
Campaign Set 2	N-L1.2.1	SRM 3666	Level I	1	Run 1	0.08	0.11	0.09	0.11	0.10	0.01	0.01	0.01	0.01	0.01
Campaign Set 2	N-L1.2.1	SRM 3666	Level I	1	Run 2	0.08	0.11	0.09	0.11	0.10	0.01	0.01	0.01	0.01	0.01
Campaign Set 2	N-L1.2.1	SRM 3666	Level I	1	Run 3	0.08	0.11	0.09	0.11	0.10	0.01	0.01	0.01	0.01	0.01
Campaign Set 2	N-L1.2.1	SRM 3666	Level I	1	Run 4	0.08	0.11	0.09	0.11	0.10	0.01	0.01	0.01	0.01	0.01
Campaign Set 2	N-L1.2.2	SRM 3666	Level I	2	Run 1	0.08	0.12	0.09	0.11	0.11	0.01	0.01	0.01	0.01	0.01
Campaign Set 2	N-L1.2.2	SRM 3666	Level I	2	Run 2	0.08	0.12	0.09	0.11	0.11	0.01	0.01	0.01	0.01	0.01
Campaign Set 2	N-L1.2.2	SRM 3666	Level I	2	Run 3	0.08	0.12	0.09	0.11	0.11	0.01	0.01	0.01	0.01	0.01
Campaign Set 2	N-L1.2.2	SRM 3666	Level I	2	Run 4	0.08	0.12	0.09	0.11	0.11	0.01	0.01	0.01	0.01	0.01
Campaign Set 2	N-L1.2.3	SRM 3666	Level I	3	Run 1	0.08	0.12	0.09	0.11	0.11	0.01	0.01	0.01	0.01	0.01
Campaign Set 2	N-L1.2.3	SRM 3666	Level I	3	Run 2	0.08	0.12	0.09	0.12	0.11	0.01	0.01	0.01	0.01	0.01
Campaign Set 2	N-L1.2.3	SRM 3666	Level I	3	Run 3	0.08	0.12	0.09	0.11	0.11	0.01	0.01	0.01	0.01	0.01
Campaign Set 2	N-L1.2.3	SRM 3666	Level I	3	Run 4	0.09	0.12	0.09	0.11	0.11	0.01	0.01	0.01	0.01	0.01
Campaign Set 2	N-L2.2.1	SRM 3666	Level II	1	Run 1	0.30	0.46	0.33	0.38	0.37	0.03	0.03	0.03	0.03	0.03
Campaign Set 2	N-L2.2.1	SRM 3666	Level II	1	Run 2	0.29	0.45	0.33	0.38	0.36	0.03	0.03	0.03	0.03	0.03

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Campaign Set 2	N-L2.2.1	SRM 3666	Level II	1	Run 3	0.30	0.46	0.33	0.38	0.36	0.03	0.03	0.03	0.03	0.03
Campaign Set 2	N-L2.2.1	SRM 3666	Level II	1	Run 4	0.31	0.45	0.33	0.38	0.36	0.03	0.03	0.03	0.03	0.03
Campaign Set 2	N-L2.2.2	SRM 3666	Level II	2	Run 1	0.27	0.42	0.29	0.34	0.33	0.03	0.03	0.03	0.03	0.03
Campaign Set 2	N-L2.2.2	SRM 3666	Level II	2	Run 2	0.26	0.41	0.30	0.34	0.33	0.03	0.03	0.03	0.03	0.03
Campaign Set 2	N-L2.2.2	SRM 3666	Level II	2	Run 3	0.28	0.41	0.30	0.34	0.32	0.03	0.03	0.03	0.03	0.03
Campaign Set 2	N-L2.2.2	SRM 3666	Level II	2	Run 4	0.27	0.41	0.29	0.33	0.33	0.03	0.03	0.03	0.02	0.03
Campaign Set 2	N-L2.2.3	SRM 3666	Level II	3	Run 1	0.31	0.50	0.35	0.40	0.38	0.03	0.04	0.03	0.03	0.03
Campaign Set 2	N-L2.2.3	SRM 3666	Level II	3	Run 2	0.31	0.47	0.35	0.40	0.38	0.03	0.04	0.03	0.03	0.03
Campaign Set 2	N-L2.2.3	SRM 3666	Level II	3	Run 3	0.31	0.48	0.35	0.40	0.38	0.03	0.04	0.03	0.03	0.03
Campaign Set 2	N-L2.2.3	SRM 3666	Level II	3	Run 4	0.31	0.48	0.35	0.40	0.39	0.03	0.04	0.03	0.03	0.03
Campaign Set 2	N-L3.2.1	SRM 3666	Level III	1	Run 1	1.12	1.24	1.12	1.20	1.14	0.11	0.11	0.11	0.11	0.11
Campaign Set 2	N-L3.2.1	SRM 3666	Level III	1	Run 2	1.13	1.24	1.12	1.22	1.16	0.11	0.11	0.11	0.11	0.11
Campaign Set 2	N-L3.2.1	SRM 3666	Level III	1	Run 3	1.10	1.24	1.11	1.24	1.13	0.11	0.11	0.11	0.11	0.11
Campaign Set 2	N-L3.2.1	SRM 3666	Level III	1	Run 4	1.09	1.23	1.12	1.24	1.14	0.10	0.11	0.11	0.11	0.11
Campaign Set 2	N-L3.2.2	SRM 3666	Level III	2	Run 1	1.06	1.16	1.06	1.14	1.09	0.10	0.10	0.10	0.10	0.10
Campaign Set 2	N-L3.2.2	SRM 3666	Level III	2	Run 2	1.05	1.18	1.04	1.14	1.08	0.10	0.10	0.10	0.10	0.10
Campaign Set 2	N-L3.2.2	SRM 3666	Level III	2	Run 3	1.07	1.18	1.05	1.14	1.07	0.10	0.10	0.10	0.10	0.10
Campaign Set 2	N-L3.2.2	SRM 3666	Level III	2	Run 4	1.04	1.17	1.04	1.12	1.09	0.10	0.10	0.10	0.09	0.10
Campaign Set 2	N-L3.2.3	SRM 3666	Level III	3	Run 1	1.09	1.20	1.07	1.16	1.11	0.10	0.10	0.10	0.10	0.10
Campaign Set 2	N-L3.2.3	SRM 3666	Level III	3	Run 2	1.08	1.22	1.06	1.19	1.11	0.10	0.10	0.10	0.10	0.10
Campaign Set 2	N-L3.2.3	SRM 3666	Level III	3	Run 3	1.11	1.19	1.08	1.16	1.10	0.11	0.10	0.10	0.10	0.10
Campaign Set 2	N-L3.2.3	SRM 3666	Level III	3	Run 4	1.07	1.20	1.07	1.20	1.12	0.10	0.10	0.10	0.10	0.10
Campaign Set 2	N-L4.2.1	SRM 3666	Level IV	1	Run 1	3.16	3.12	3.00	3.13	2.86	0.36	0.37	0.37	0.37	0.36
Campaign Set 2	N-L4.2.1	SRM 3666	Level IV	1	Run 2	3.14	3.12	3.01	3.19	2.96	0.36	0.37	0.37	0.37	0.37
Campaign Set 2	N-L4.2.1	SRM 3666	Level IV	1	Run 3	3.16	3.13	2.99	3.12	2.99	0.36	0.37	0.37	0.37	0.38
Campaign Set 2	N-L4.2.1	SRM 3666	Level IV	1	Run 4	3.16	3.12	3.02	3.16	2.96	0.36	0.37	0.37	0.37	0.37
Campaign Set 2	N-L4.2.2	SRM 3666	Level IV	2	Run 1	3.29	3.07	2.99	3.15	2.89	0.36	0.35	0.35	0.36	0.35
Campaign Set 2	N-L4.2.2	SRM 3666	Level IV	2	Run 2	3.21	3.12	2.98	3.11	2.92	0.35	0.35	0.35	0.35	0.35
Campaign Set 2	N-L4.2.2	SRM 3666	Level IV	2	Run 3	3.11	3.12	2.99	3.11	2.90	0.34	0.36	0.35	0.35	0.35
Campaign Set 2	N-L4.2.2	SRM 3666	Level IV	2	Run 4	3.13	3.08	2.97	3.16	2.90	0.34	0.35	0.35	0.36	0.35
Campaign Set 2	N-L4.2.3	SRM 3666	Level IV	3	Run 1	3.14	3.04	2.94	3.07	2.81	0.35	0.34	0.34	0.35	0.34
Campaign Set 2	N-L4.2.3	SRM 3666	Level IV	3	Run 2	3.07	3.05	2.96	3.04	2.90	0.34	0.35	0.34	0.34	0.35
Campaign Set 2	N-L4.2.3	SRM 3666	Level IV	3	Run 3	3.07	3.05	2.96	3.09	2.89	0.34	0.35	0.34	0.35	0.35
Campaign Set 2	N-L4.2.3	SRM 3666	Level IV	3	Run 4	3.07	3.06	2.97	3.05	2.89	0.34	0.35	0.34	0.34	0.35
Campaign Set 2	S-L1.2.1	HRM 3004A-01	Level 1	1	Run 1	0.41	0.52	0.44	0.49	0.47	0.04	0.04	0.04	0.04	0.04
Campaign Set 2	S-L1.2.1	HRM 3004A-01	Level 1	1	Run 2	0.40	0.52	0.44	0.49	0.47	0.04	0.04	0.04	0.04	0.04
Campaign Set 2	S-L1.2.1	HRM 3004A-01	Level 1	1	Run 3	0.40	0.52	0.43	0.49	0.47	0.04	0.04	0.04	0.04	0.04
Campaign Set 2	S-L1.2.1	HRM 3004A-01	Level 1	1	Run 4	0.39	0.52	0.44	0.49	0.47	0.04	0.04	0.04	0.04	0.04
Campaign Set 2	S-L1.2.2	HRM 3004A-01	Level 1	2	Run 1	0.39	0.50	0.41	0.48	0.45	0.04	0.04	0.04	0.04	0.04
Campaign Set 2	S-L1.2.2	HRM 3004A-01	Level 1	2	Run 2	0.39	0.49	0.42	0.48	0.45	0.04	0.04	0.04	0.04	0.04
Campaign Set 2	S-L1.2.2	HRM 3004A-01	Level 1	2	Run 3	0.37	0.49	0.41	0.47	0.45	0.04	0.04	0.04	0.04	0.04
Campaign Set 2	S-L1.2.2	HRM 3004A-01	Level 1	2	Run 4	0.38	0.49	0.41	0.47	0.45	0.04	0.04	0.04	0.04	0.04
Campaign Set 2	S-L1.2.3	HRM 3004A-01	Level 1	3	Run 1	0.41	0.51	0.42	0.48	0.46	0.04	0.04	0.04	0.04	0.04
Campaign Set 2	S-L1.2.3	HRM 3004A-01	Level 1	3	Run 2	0.39	0.51	0.41	0.48	0.46	0.04	0.04	0.04	0.04	0.04
Campaign Set 2	S-L1.2.3	HRM 3004A-01	Level 1	3	Run 3	0.39	0.51	0.43	0.49	0.46	0.04	0.04	0.04	0.04	0.04
Campaign Set 2	S-L1.2.3	HRM 3004A-01	Level 1	3	Run 4	0.40	0.50	0.43	0.49	0.46	0.04	0.04	0.04	0.04	0.04

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Campaign Set 2	S-L2.2.1	HRM 3004A-02	Level 2	1	Run 1	2.01	2.00	1.89	1.99	1.90	0.22	0.22	0.22	0.22	0.22
Campaign Set 2	S-L2.2.1	HRM 3004A-02	Level 2	1	Run 2	1.97	2.02	1.89	2.03	1.88	0.22	0.22	0.22	0.22	0.22
Campaign Set 2	S-L2.2.1	HRM 3004A-02	Level 2	1	Run 3	1.95	2.03	1.89	2.03	1.90	0.22	0.22	0.22	0.22	0.22
Campaign Set 2	S-L2.2.1	HRM 3004A-02	Level 2	1	Run 4	1.94	2.01	1.90	2.03	1.89	0.21	0.22	0.22	0.22	0.22
Campaign Set 2	S-L2.2.2	HRM 3004A-02	Level 2	2	Run 1	1.96	1.98	1.89	1.99	1.87	0.22	0.22	0.22	0.22	0.22
Campaign Set 2	S-L2.2.2	HRM 3004A-02	Level 2	2	Run 2	1.91	2.03	1.88	2.01	1.90	0.21	0.22	0.22	0.22	0.22
Campaign Set 2	S-L2.2.2	HRM 3004A-02	Level 2	2	Run 3	1.98	2.02	1.86	2.05	1.91	0.22	0.22	0.22	0.22	0.22
Campaign Set 2	S-L2.2.2	HRM 3004A-02	Level 2	2	Run 4	1.94	2.03	1.88	2.01	1.94	0.22	0.22	0.22	0.22	0.23
Campaign Set 2	S-L2.2.3	HRM 3004A-02	Level 2	3	Run 1	1.92	2.03	1.88	1.99	1.88	0.21	0.22	0.22	0.22	0.22
Campaign Set 2	S-L2.2.3	HRM 3004A-02	Level 2	3	Run 2	1.97	2.02	1.93	2.00	1.90	0.22	0.22	0.23	0.22	0.22
Campaign Set 2	S-L2.2.3	HRM 3004A-02	Level 2	3	Run 3	1.90	2.02	1.88	1.99	1.88	0.21	0.22	0.22	0.22	0.22
Campaign Set 2	S-L2.2.3	HRM 3004A-02	Level 2	3	Run 4	1.93	2.01	1.91	2.04	1.89	0.22	0.22	0.22	0.22	0.22

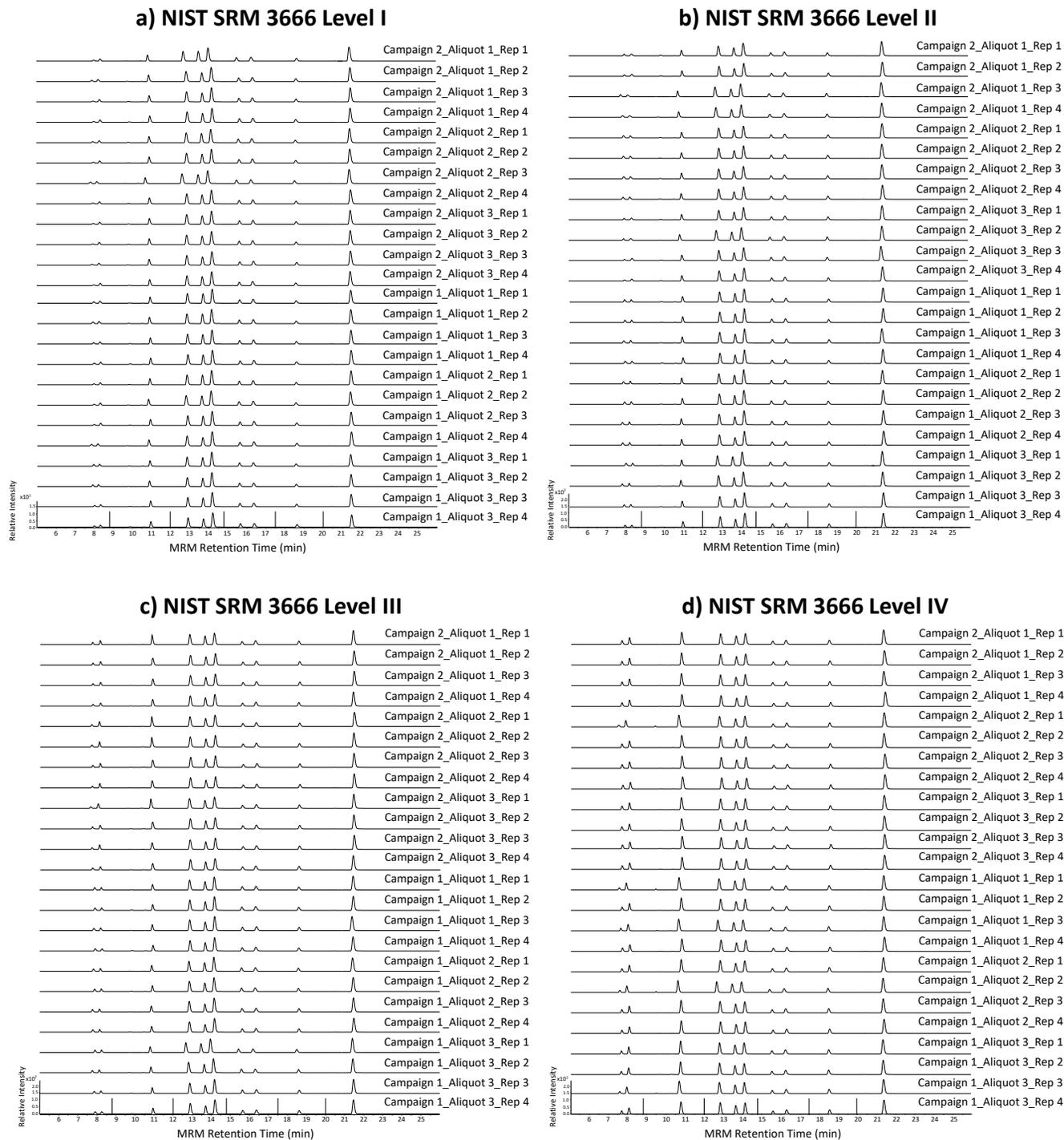


Fig. A1. MRM chromatographic profiles of NIST SRM 3666 samples [Level I (a), Level II (b), Level III (c), and Level IV (d)].

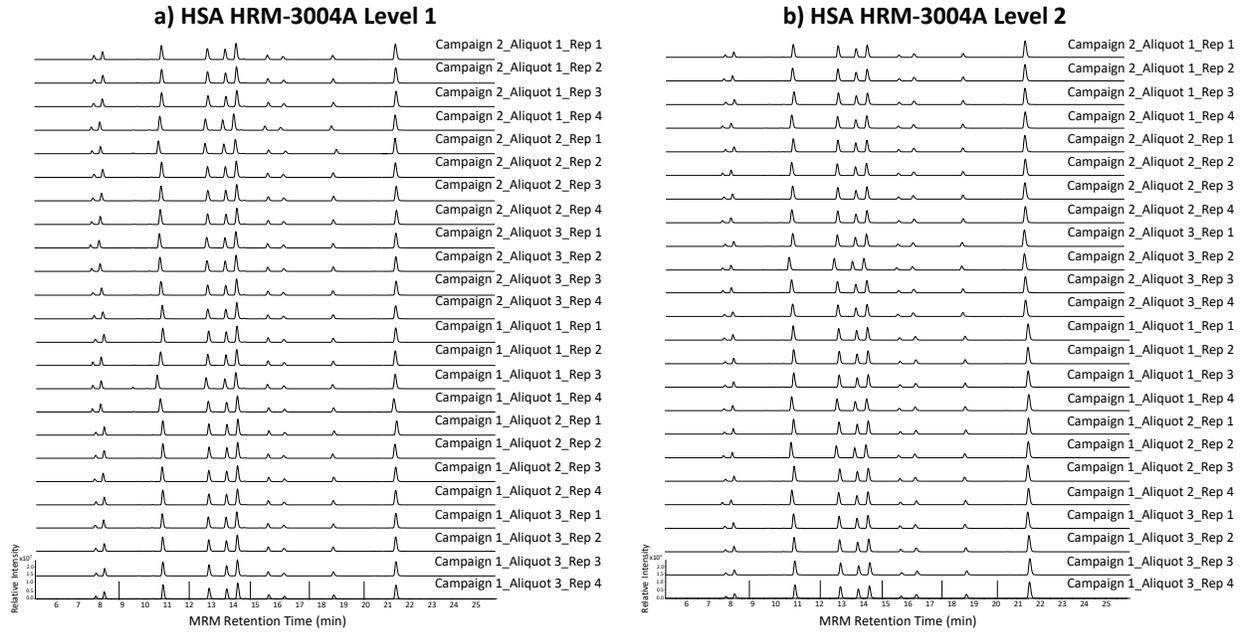


Fig. A2. MRM chromatographic profiles of HSA HRM-3004A samples [Level 1 (a) and Level 2 (b)].

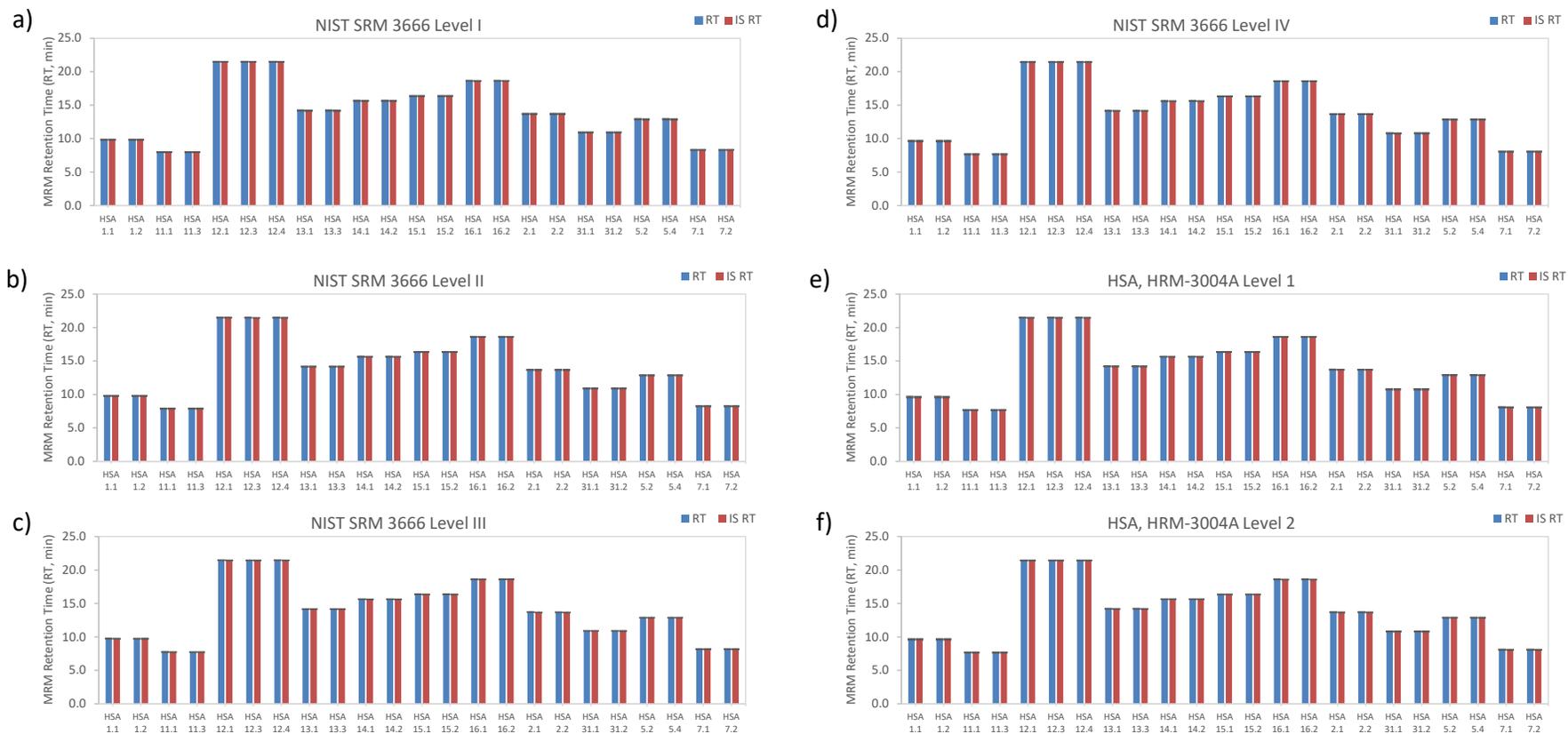


Fig. A3. Urine albumin MRM retention time (RT) profile (n = 24) of NIST SRM 3666 (Level I to Level IV) and HSA HRM-3004A (Level 1 and Level 2) extent-of-equivalence samples.

The error bars represent the standard error of the mean (n = 24) for the combined RT and IS RT results. [SRM 3666 Level I (a), SRM 3666 Level II (b), SRM 3666 Level III (c), SRM 3666 Level IV (d), HRM-3004A Level 1 (e), HRM-3004A (f)].

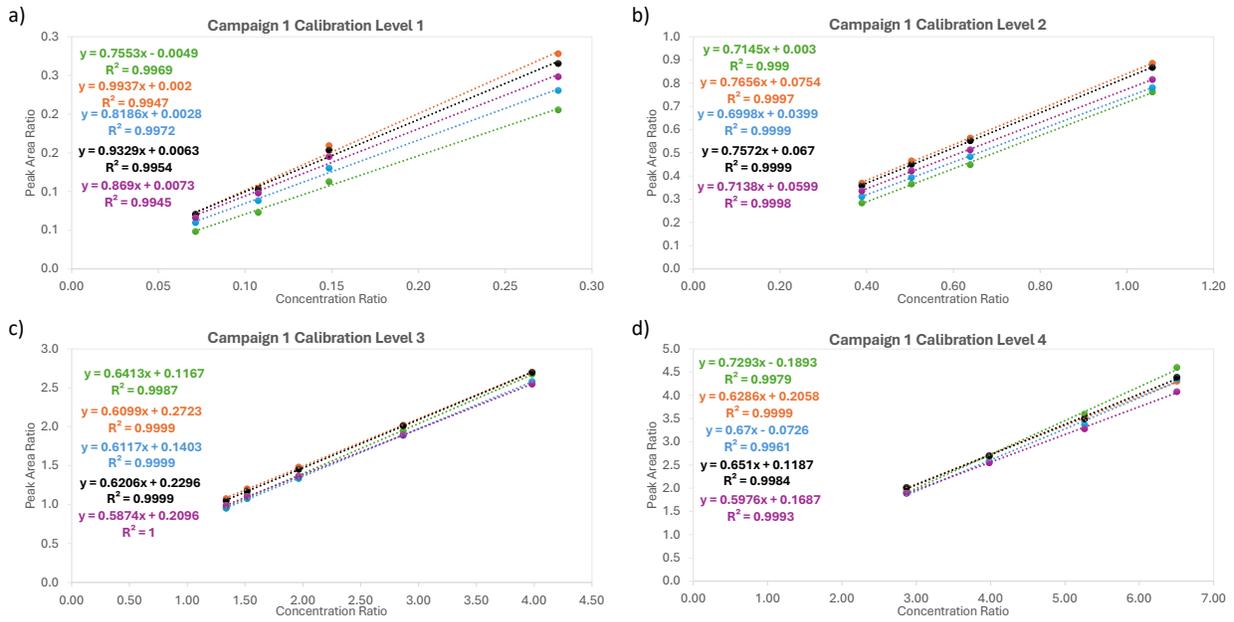


Fig. A4. Bracketed calibration curves (n = 4) of the five (5) qt-MRM Transitions [HSA 11.1 (green), HSA 12.4 (orange), HSA 13.3 (blue), HSA 31.1 (black), and HSA 5.2 (purple)] used in the Campaign 1 Set: Calibration Level 1 (a), Calibration Level 2 (b), Calibration Level 3 (c), and Calibration Level 4 (d) (refer to Table A1 for the urine albumin concentration ranges used in each calibration level).

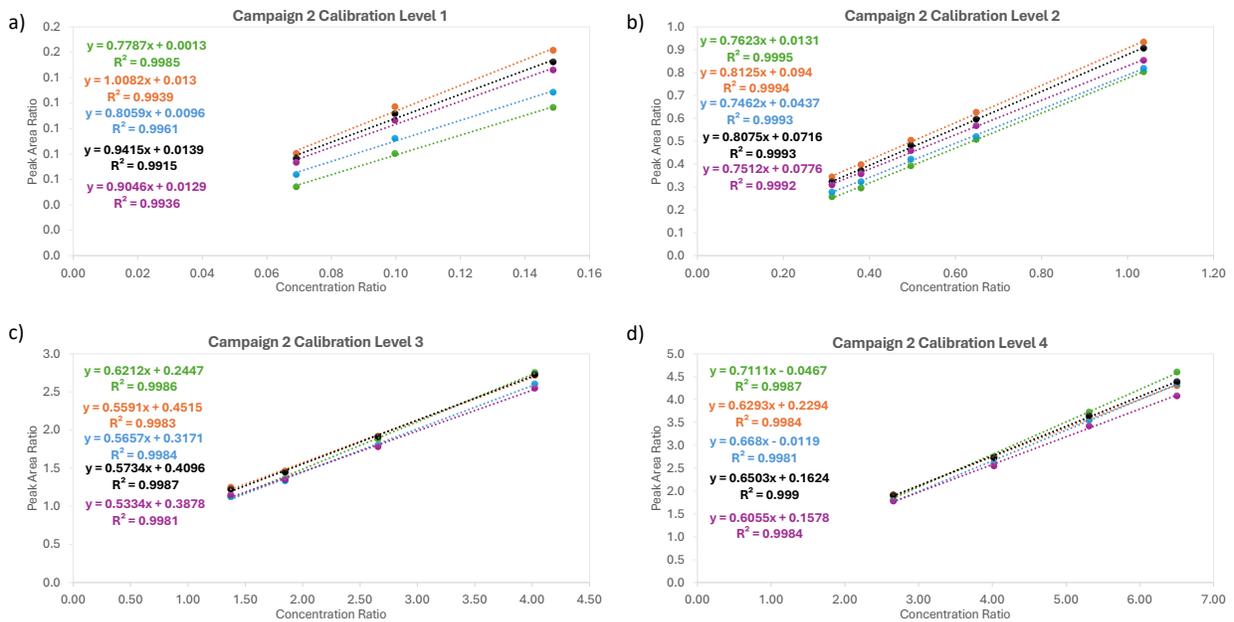


Fig. A5. Bracketed calibration curves (n = 4) of the five (5) qt-MRM Transitions [HSA 11.1 (green), HSA 12.4 (orange), HSA 13.3 (blue), HSA 31.1 (black), and HSA 5.2 (purple)] used in the Campaign 2 Set: Calibration Level 1 (a), Calibration Level 2 (b), Calibration Level 3 (c), and Calibration Level 4 (d) (refer to Table A1 for the urine albumin concentration ranges used in each calibration level).

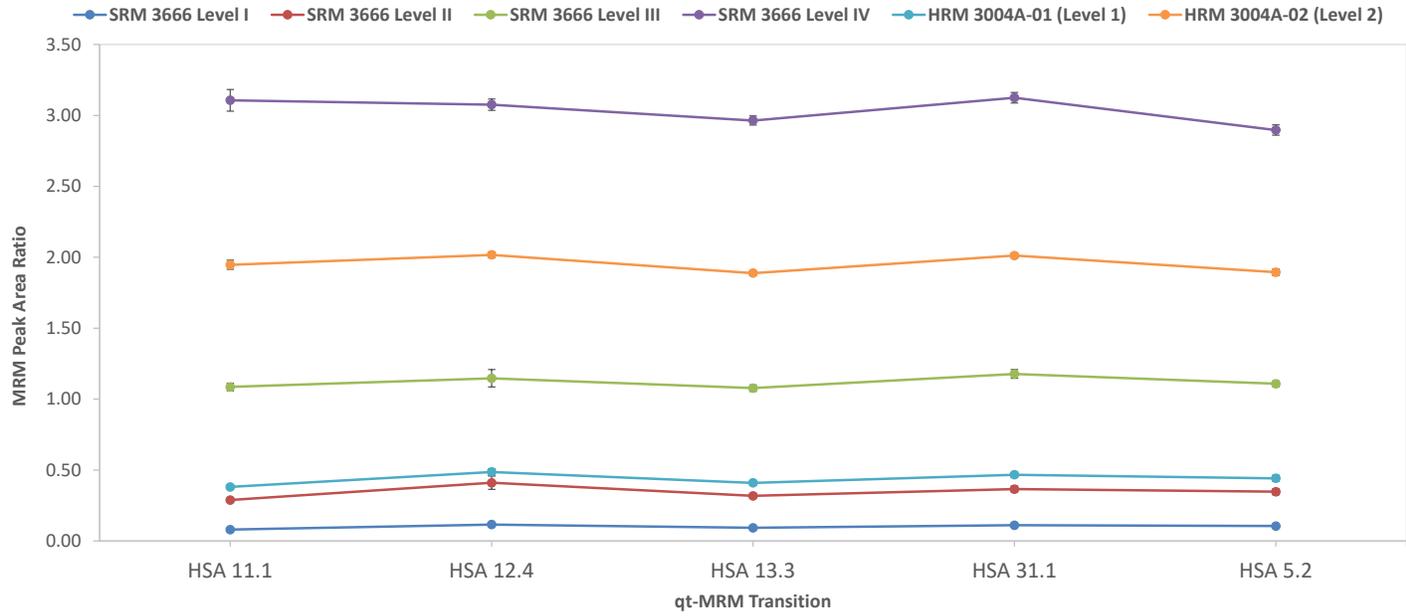


Fig. A6. MRM peak area ratio (PAR) profile of the qt-MRM Transitions for the NIST SRM 3666 (Level I to Level IV) and HSA HRM-3004A (Level 1 and Level 2) extent-of-equivalence samples.

The error bars represent the standard error of the mean ($n = 24$) for the combined PAR results [Campaigns (2), Aliquots (6), and Technical Replicates (24)].

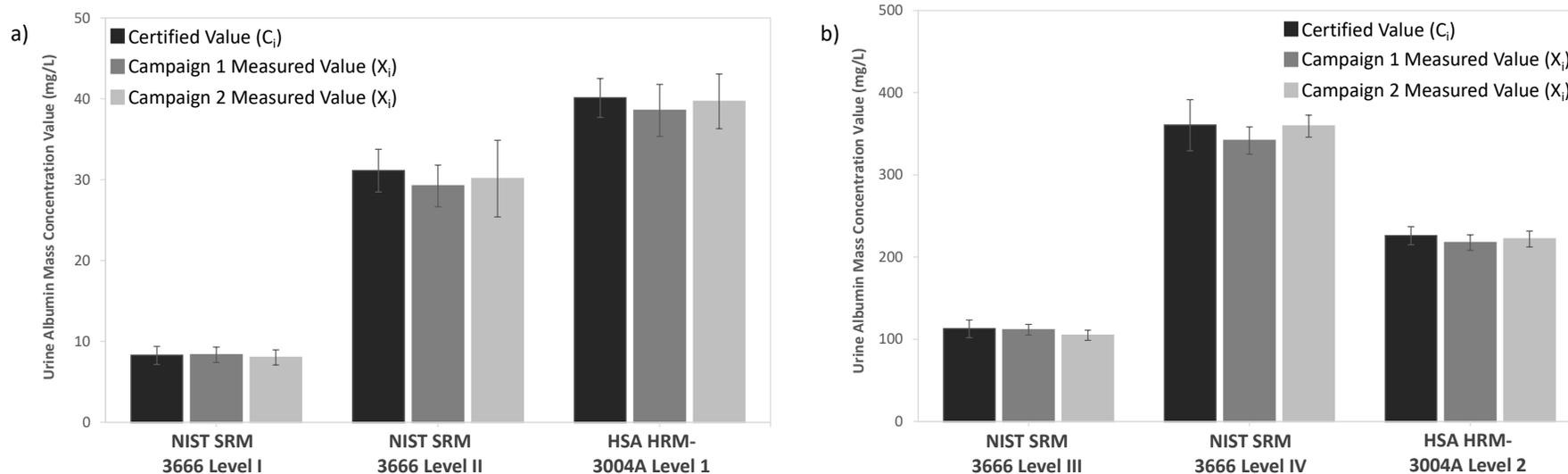


Fig. A7. Plots of certified value versus measured consensus mass concentration ($\bar{X}_{mg/L}$) values for urine albumin for the CRM samples: NIST SRM 3666 Level I (a), NIST SRM 3666 Level II (b), NIST SRM 3666 Level III (b), NIST SRM 3666 Level IV (b), HSA HRM-3004A Level 1 (a), and HSA HRM-3004A Level 2 (b).

The error bars for the certified value represent the expanded uncertainty ($k = 2$) of the certified value and the error bars for the measured values represent the expanded uncertainty ($k = 2$) of the $\bar{X}_{mg/L}$ values ($n = 60$) for each sample.

APPENDIX B – URINE CREATININE EXTENT-OF-EQUIVALENCE MEASUREMENTS

Supplemental Data and Graphs: Extent-of-Equivalence Analysis for Urine Creatinine Measurements in NIST SRM 3666 and HSA HRM-30304A

Table B1. Extent-of-equivalence samples for urine creatinine measurements.

Campaign Set	Campaign (Unit)	NMI	CRM Name	Level	Aliquot#	Creatinine Sample Label Name
Campaign Set 1	1	NIST	NIST SRM 3666	Level I	1	SRM3666 L1V1sx1
Campaign Set 1	1	NIST	NIST SRM 3666	Level I	2	SRM3666 L1V1sx2
Campaign Set 1	1	NIST	NIST SRM 3666	Level I	3	SRM3666 L1V1sx3
Campaign Set 1	1	NIST	NIST SRM 3666	Level II	1	SRM3666 L2V1sx1
Campaign Set 1	1	NIST	NIST SRM 3666	Level II	2	SRM3666 L2V1sx2
Campaign Set 1	1	NIST	NIST SRM 3666	Level II	3	SRM3666 L2V1sx3
Campaign Set 1	1	NIST	NIST SRM 3666	Level III	1	SRM3666 L3V1sx1
Campaign Set 1	1	NIST	NIST SRM 3666	Level III	2	SRM3666 L3V1sx2
Campaign Set 1	1	NIST	NIST SRM 3666	Level III	3	SRM3666 L3V1sx3
Campaign Set 1	1	NIST	NIST SRM 3666	Level IV	1	SRM3666 L4V1sx1
Campaign Set 1	1	NIST	NIST SRM 3666	Level IV	2	SRM3666 L4V1sx2
Campaign Set 1	1	NIST	NIST SRM 3666	Level IV	3	SRM3666 L4V1sx3
Campaign Set 1	1	HSA, Singapore	HRM-3004A	Level 1	1	HRM-3004A L1V1sx1
Campaign Set 1	1	HSA, Singapore	HRM-3004A	Level 1	2	HRM-3004A L1V1sx2
Campaign Set 1	1	HSA, Singapore	HRM-3004A	Level 1	3	HRM-3004A L1V1sx3
Campaign Set 1	1	HSA, Singapore	HRM-3004A	Level 2	1	HRM-3004A L2V1sx1
Campaign Set 1	1	HSA, Singapore	HRM-3004A	Level 2	2	HRM-3004A L2V1sx2
Campaign Set 1	1	HSA, Singapore	HRM-3004A	Level 2	3	HRM-3004A L2V1sx3
Campaign Set 2	2	NIST	NIST SRM 3666	Level I	1	SRM3666 L1V2sx1
Campaign Set 2	2	NIST	NIST SRM 3666	Level I	2	SRM3666 L1V2sx2
Campaign Set 2	2	NIST	NIST SRM 3666	Level I	3	SRM3666 L1V2sx3
Campaign Set 2	2	NIST	NIST SRM 3666	Level II	1	SRM3666 L2V2sx1
Campaign Set 2	2	NIST	NIST SRM 3666	Level II	2	SRM3666 L2V2sx2
Campaign Set 2	2	NIST	NIST SRM 3666	Level II	3	SRM3666 L2V2sx3
Campaign Set 2	2	NIST	NIST SRM 3666	Level III	1	SRM3666 L3V2sx1
Campaign Set 2	2	NIST	NIST SRM 3666	Level III	2	SRM3666 L3V2sx2
Campaign Set 2	2	NIST	NIST SRM 3666	Level III	3	SRM3666 L3V2sx3
Campaign Set 2	2	NIST	NIST SRM 3666	Level IV	1	SRM3666 L4V2sx1
Campaign Set 2	2	NIST	NIST SRM 3666	Level IV	2	SRM3666 L4V2sx2
Campaign Set 2	2	NIST	NIST SRM 3666	Level IV	3	SRM3666 L4V2sx3
Campaign Set 2	2	HSA, Singapore	HRM-3004A	Level 1	1	HRM-3004A L1V2sx1
Campaign Set 2	2	HSA, Singapore	HRM-3004A	Level 1	2	HRM-3004A L1V2sx2
Campaign Set 2	2	HSA, Singapore	HRM-3004A	Level 1	3	HRM-3004A L1V2sx3
Campaign Set 2	2	HSA, Singapore	HRM-3004A	Level 2	1	HRM-3004A L2V2sx1
Campaign Set 2	2	HSA, Singapore	HRM-3004A	Level 2	2	HRM-3004A L2V2sx2
Campaign Set 2	2	HSA, Singapore	HRM-3004A	Level 2	3	HRM-3004A L2V2sx3

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Campaign Set 1	SRM3666 L4V1sx2 (1to100D)_08062025	5.0	Campaign Set 2	HRM-3004A L2V2sx2 (1to100D)_08112025	5.0
Campaign Set 1	SRM3666 L1V1sx3 (1to100D)_08062025	5.0	Campaign Set 2	SRM3666 L1V2sx3 (1to100D)_08112025	5.0
Campaign Set 1	HRM-3004A L2V1sx1 (1to100D)_08062025	5.0	Campaign Set 2	SRM3666 L3V2sx3 (1to100D)_08112025	5.0
Campaign Set 1	SRM3666 L3V1sx1 (1to100D)_08062025	5.0	Campaign Set 2	HRM-3004A L2V2sx1 (1to100D)_08112025	5.0
Campaign Set 1	SRM3666 L4V1sx3 (1to100D)_08062025	5.0	Campaign Set 2	SRM3666 L4V2sx3 (1to100D)_08112025	5.0
Campaign Set 1	SRM3666 L2V1sx2 (1to100D)_08062025	5.0	Campaign Set 2	SRM3666 L2V2sx3 (1to100D)_08112025	5.0
Campaign Set 1	HRM-3004A L1V1sx1 (1to100D)_08062025	5.0	Campaign Set 2	HRM-3004A L1V2sx2 (1to100D)_08112025	5.0
Campaign Set 1	SRM3666 L3V1sx2 (1to100D)_08062025	5.0	Campaign Set 2	SRM3666 L3V2sx2 (1to100D)_08112025	5.0
Campaign Set 1	SRM3667 V1sx3 (1to100D)_08062025	5.0	Campaign Set 2	SRM3666 L4V2sx1 (1to100D)_08112025	5.0
Campaign Set 1	SRM3666 L3V1sx3 (1to100D)_08062025	5.0	Campaign Set 2	HRM-3004A L1V2sx3 (1to100D)_08112025	5.0
Campaign Set 1	SRM3666 L4V1sx1 (1to100D)_08062025	5.0	Campaign Set 2	SRM3666 L2V2sx1 (1to100D)_08112025	5.0
Campaign Set 1	SRM3666 L1V1sx2 (1to100D)_08062025	5.0	Campaign Set 2	SRM3666 L4V2sx2 (1to100D)_08112025	5.0
Campaign Set 1	SRM3666 L2V1sx3 (1to100D)_08062025	5.0	Campaign Set 2	SRM3667 V2sx3 (1to100D)_08112025	5.0
Campaign Set 1	HRM-3004A L1V1sx2 (1to100D)_08062025	5.0	Campaign Set 2	SRM3666 L2V2sx2 (1to100D)_08112025	5.0
Campaign Set 1	SRM3666 L2V1sx1 (1to100D)_08062025	5.0	Campaign Set 2	HRM-3004A L1V2sx1 (1to100D)_08112025	5.0
Campaign Set 1	HRM-3004A L1V1sx3 (1to100D)_08062025	5.0	Campaign Set 2	SRM3666 L3V2sx1 (1to100D)_08112025	5.0
Campaign Set 1	HRM-3004A L2V1sx3 (1to100D)_08062025	5.0	Campaign Set 2	SRM3666 L1V2sx2 (1to100D)_08112025	5.0
Campaign Set 1	SRM3667 V1sx2 (1to100D)_08062025	5.0	Campaign Set 2	SRM3667 V2sx2 (1to100D)_08112025	5.0
Campaign Set 1	0.01M HCl blank	5.0	Campaign Set 2	0.01M HCl blank	5.0
Campaign Set 1	Cal 5 (1to50D)-08062025	5.0	Campaign Set 2	Cal 3 (1to50D)-08112025	5.0
Campaign Set 1	Cal 1 (1to50D)-08062025	5.0	Campaign Set 2	Cal 5 (1to50D)-08112025	5.0
Campaign Set 1	Cal 3 (1to50D)-08062025	5.0	Campaign Set 2	Cal 4 (1to50D)-08112025	5.0
Campaign Set 1	Cal 2 (1to50D)-08062025	5.0	Campaign Set 2	Cal 1 (1to50D)-08112025	5.0
Campaign Set 1	Cal 4 (1to50D)-08062025	5.0	Campaign Set 2	Cal 2 (1to50D)-08112025	5.0
Campaign Set 1	0.01M HCl blank	5.0	Campaign Set 2	0.01M HCl blank	5.0

Table B3. Peak area ratio (PAR) and relative response factor (RRF) values for calibration samples.

Campaign Set	Sample Name	Technical Replicate	Peak Area (m/z 114)	Peak Area (m/z 117)	Peak Area Ratio (PAR)	Relative Response Factor (RRF)	Calibration Summary Statistics	
Campaign Set 1	Calibrant 1	1	229270.5	282627.9	0.81121	1.01772	1.01211	Avg. RFF (n=15)
Campaign Set 1	Calibrant 1	2	238013.7	290386.9	0.81964	1.02830	0.00878	Stdev
Campaign Set 1	Calibrant 1	3	241782.9	298191.0	0.81083	1.01724	0.87	%CV
Campaign Set 1	Calibrant 2	1	258152.2	281230.9	0.91794	1.02189		
Campaign Set 1	Calibrant 2	2	266831.3	289498.0	0.92170	1.02609		
Campaign Set 1	Calibrant 2	3	270534.6	296346.1	0.91290	1.01629		
Campaign Set 1	Calibrant 3	1	282174.6	278411.5	1.01352	1.01195		
Campaign Set 1	Calibrant 3	2	290259.7	287121.3	1.01093	1.00937		
Campaign Set 1	Calibrant 3	3	297231.8	295398.9	1.00620	1.00465		
Campaign Set 1	Calibrant 4	1	314034.8	281911.0	1.11395	1.00642		
Campaign Set 1	Calibrant 4	2	323210.9	290915.3	1.11101	1.00377		
Campaign Set 1	Calibrant 4	3	332087.4	298399.6	1.11289	1.00547		
Campaign Set 1	Calibrant 5	1	340002.1	283336.9	1.19999	1.00783		
Campaign Set 1	Calibrant 5	2	345592.2	290715.3	1.18877	0.99840		
Campaign Set 1	Calibrant 5	3	356956.2	297949.4	1.19804	1.00619		
Campaign Set 2	Calibrant 1	1	217767.6	268501.1	0.81105	1.01452	1.00471	Avg. RFF (n=15)
Campaign Set 2	Calibrant 1	2	230093.1	285210.8	0.80675	1.00914	0.00779	Stdev
Campaign Set 2	Calibrant 1	3	235690.9	291414.3	0.80878	1.01169	0.78	%CV
Campaign Set 2	Calibrant 2	1	248853.6	272892.4	0.91191	1.01301		
Campaign Set 2	Calibrant 2	2	261495.5	285534.1	0.91581	1.01734		
Campaign Set 2	Calibrant 2	3	265543.0	291892.2	0.90973	1.01059		
Campaign Set 2	Calibrant 3	1	278657.4	278193.3	1.00167	1.00134		
Campaign Set 2	Calibrant 3	2	292101.8	290871.3	1.00423	1.00390		
Campaign Set 2	Calibrant 3	3	299607.1	297691.9	1.00643	1.00611		
Campaign Set 2	Calibrant 4	1	296941.2	267719.0	1.10915	1.00113		
Campaign Set 2	Calibrant 4	2	304146.9	274941.2	1.10623	0.99849		
Campaign Set 2	Calibrant 4	3	311810.6	282627.1	1.10326	0.99581		
Campaign Set 1	Calibrant 5	1	324953.0	272169.3	1.19394	1.00025		
Campaign Set 1	Calibrant 5	2	331314.7	278626.4	1.18910	0.99620		
Campaign Set 1	Calibrant 5	3	339978.3	287384.4	1.18301	0.99110		

Table B4. Peak area ratio (PAR) and mass fraction ($\mu\text{g/g}$) values for urine creatinine CRM samples.

Campaign Set	Institute	CRM Name	Level	Campaign (Vial)	Aliquot	Technical Replicate	Peak Area (m/z 114)	Peak Area (m/z 117)	Peak Area Ratio (PAR)	Mass Fraction ($\mu\text{g/g}$)
Campaign Set 1	NIST	NIST SRM 3666	Level I	1	1	Run 1	306244.2	306116.2	1.00042	1148.16921
Campaign Set 1	NIST	NIST SRM 3666	Level I	1	1	Run 2	311819.1	311693.3	1.00040	1148.15252
Campaign Set 1	NIST	NIST SRM 3666	Level I	1	1	Run 3	315881.4	316555.7	0.99787	1145.24460
Campaign Set 1	NIST	NIST SRM 3666	Level I	1	2	Run 1	329521.6	327009.7	1.00768	1150.00029
Campaign Set 1	NIST	NIST SRM 3666	Level I	1	2	Run 2	332252.5	329627.8	1.00796	1150.32120
Campaign Set 1	NIST	NIST SRM 3666	Level I	1	2	Run 3	337664.0	336728.3	1.00278	1144.40525
Campaign Set 1	NIST	NIST SRM 3666	Level I	1	3	Run 1	312903.0	311955.2	1.00304	1147.86023
Campaign Set 1	NIST	NIST SRM 3666	Level I	1	3	Run 2	318743.5	316624.4	1.00669	1152.04242
Campaign Set 1	NIST	NIST SRM 3666	Level I	1	3	Run 3	318750.8	319042.5	0.99909	1143.33699
Campaign Set 1	NIST	NIST SRM 3666	Level II	1	1	Run 1	304549.3	306908.4	0.99231	1179.52681
Campaign Set 1	NIST	NIST SRM 3666	Level II	1	1	Run 2	306031.5	309587.0	0.98852	1175.01227
Campaign Set 1	NIST	NIST SRM 3666	Level II	1	1	Run 3	312979.1	315889.2	0.99079	1177.71321
Campaign Set 1	NIST	NIST SRM 3666	Level II	1	2	Run 1	311662.4	316285.3	0.98538	1166.05359
Campaign Set 1	NIST	NIST SRM 3666	Level II	1	2	Run 2	314359.7	318630.2	0.98660	1167.48964
Campaign Set 1	NIST	NIST SRM 3666	Level II	1	2	Run 3	317972.6	322782.9	0.98510	1165.71473
Campaign Set 1	NIST	NIST SRM 3666	Level II	1	3	Run 1	314285.6	318714.3	0.98610	1167.87107
Campaign Set 1	NIST	NIST SRM 3666	Level II	1	3	Run 2	315655.5	321535.3	0.98171	1162.67054
Campaign Set 1	NIST	NIST SRM 3666	Level II	1	3	Run 3	320770.3	328864.5	0.97539	1155.17856
Campaign Set 1	NIST	NIST SRM 3666	Level III	1	1	Run 1	318301.8	307205.7	1.03612	1234.45145
Campaign Set 1	NIST	NIST SRM 3666	Level III	1	1	Run 2	321995.2	309570.3	1.04014	1239.23680
Campaign Set 1	NIST	NIST SRM 3666	Level III	1	1	Run 3	325353.1	314125.8	1.03574	1234.00106
Campaign Set 1	NIST	NIST SRM 3666	Level III	1	2	Run 1	322223.5	313456.5	1.02797	1253.46479
Campaign Set 1	NIST	NIST SRM 3666	Level III	1	2	Run 2	323361.7	314815.0	1.02715	1252.46435
Campaign Set 1	NIST	NIST SRM 3666	Level III	1	2	Run 3	327840.9	320755.1	1.02209	1246.29764
Campaign Set 1	NIST	NIST SRM 3666	Level III	1	3	Run 1	321533.0	315699.4	1.01848	1247.98403
Campaign Set 1	NIST	NIST SRM 3666	Level III	1	3	Run 2	324540.5	318406.1	1.01927	1248.94914
Campaign Set 1	NIST	NIST SRM 3666	Level III	1	3	Run 3	327249.6	323698.3	1.01097	1238.78500
Campaign Set 1	NIST	NIST SRM 3666	Level IV	1	1	Run 1	304731.6	307197.5	0.99197	1248.25495
Campaign Set 1	NIST	NIST SRM 3666	Level IV	1	1	Run 2	305199.9	307672.3	0.99196	1248.24396
Campaign Set 1	NIST	NIST SRM 3666	Level IV	1	1	Run 3	310573.9	312995.5	0.99226	1248.62017
Campaign Set 1	NIST	NIST SRM 3666	Level IV	1	2	Run 1	305095.7	308273.2	0.98969	1253.67545
Campaign Set 1	NIST	NIST SRM 3666	Level IV	1	2	Run 2	309144.3	312290.5	0.98993	1253.97037
Campaign Set 1	NIST	NIST SRM 3666	Level IV	1	2	Run 3	309003.6	313954.6	0.98423	1246.75607
Campaign Set 1	NIST	NIST SRM 3666	Level IV	1	3	Run 1	311740.8	312384.6	0.99794	1252.42402
Campaign Set 1	NIST	NIST SRM 3666	Level IV	1	3	Run 2	312176.8	313482.3	0.99584	1249.78400
Campaign Set 1	NIST	NIST SRM 3666	Level IV	1	3	Run 3	315409.9	315623.5	0.99932	1254.16117
Campaign Set 1	HSA, Singapore	HRM 3004A-01	Level 1	1	1	Run 1	328862.7	328400.2	1.00141	394.47900
Campaign Set 1	HSA, Singapore	HRM 3004A-01	Level 1	1	1	Run 2	328193.3	328946.4	0.99771	393.02236
Campaign Set 1	HSA, Singapore	HRM 3004A-01	Level 1	1	1	Run 3	332496.7	333576.7	0.99676	392.64884
Campaign Set 1	HSA, Singapore	HRM 3004A-01	Level 1	1	2	Run 1	340281.0	335243.4	1.01503	396.91399
Campaign Set 1	HSA, Singapore	HRM 3004A-01	Level 1	1	2	Run 2	340084.9	339364.8	1.00212	391.86773
Campaign Set 1	HSA, Singapore	HRM 3004A-01	Level 1	1	2	Run 3	347204.5	344569.0	1.00765	394.02891
Campaign Set 1	HSA, Singapore	HRM 3004A-01	Level 1	1	3	Run 1	335837.6	333842.3	1.00598	396.61350
Campaign Set 1	HSA, Singapore	HRM 3004A-01	Level 1	1	3	Run 2	336966.7	336528.0	1.00130	394.77107
Campaign Set 1	HSA, Singapore	HRM 3004A-01	Level 1	1	3	Run 3	343397.6	344237.7	0.99756	393.29494
Campaign Set 1	HSA, Singapore	HRM 3004A-02	Level 2	1	1	Run 1	315642.8	308051.4	1.02464	1204.10037
Campaign Set 1	HSA, Singapore	HRM 3004A-02	Level 2	1	1	Run 2	315712.6	308284.0	1.02410	1203.45795
Campaign Set 1	HSA, Singapore	HRM 3004A-02	Level 2	1	1	Run 3	318512.9	310721.3	1.02508	1204.60869
Campaign Set 1	HSA, Singapore	HRM 3004A-02	Level 2	1	2	Run 1	318917.2	311336.1	1.02435	1206.14817
Campaign Set 1	HSA, Singapore	HRM 3004A-02	Level 2	1	2	Run 2	317658.3	313902.6	1.01196	1191.56433
Campaign Set 1	HSA, Singapore	HRM 3004A-02	Level 2	1	2	Run 3	320730.5	314640.0	1.01936	1200.26883
Campaign Set 1	HSA, Singapore	HRM 3004A-02	Level 2	1	3	Run 1	316992.9	314649.3	1.00745	1194.11171
Campaign Set 1	HSA, Singapore	HRM 3004A-02	Level 2	1	3	Run 2	316933.4	315338.4	1.00506	1191.27861
Campaign Set 1	HSA, Singapore	HRM 3004A-02	Level 2	1	3	Run 3	324207.7	320926.4	1.01022	1197.40226
Campaign Set 2	NIST	NIST SRM 3666	Level I	2	1	Run 1	267698.1	277200.0	0.96572	1130.42828
Campaign Set 2	NIST	NIST SRM 3666	Level I	2	1	Run 2	271533.3	282873.5	0.95991	1123.62601
Campaign Set 2	NIST	NIST SRM 3666	Level I	2	1	Run 3	274924.0	286227.9	0.96051	1124.32441

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Campaign Set 2	NIST	NIST SRM 3666	Level I	2	2	Run 1	277821.8	284301.8	0.97721	1131.78678
Campaign Set 2	NIST	NIST SRM 3666	Level I	2	2	Run 2	282024.9	290426.0	0.97107	1124.68232
Campaign Set 2	NIST	NIST SRM 3666	Level I	2	2	Run 3	287785.8	296558.8	0.97042	1123.92272
Campaign Set 2	NIST	NIST SRM 3666	Level I	2	3	Run 1	279461.7	287349.9	0.97255	1126.63039
Campaign Set 2	NIST	NIST SRM 3666	Level I	2	3	Run 2	283639.4	291688.5	0.97241	1126.46439
Campaign Set 2	NIST	NIST SRM 3666	Level I	2	3	Run 3	287838.5	294952.4	0.97588	1130.49115
Campaign Set 2	NIST	NIST SRM 3666	Level II	2	1	Run 1	281660.7	292320.6	0.96353	1128.44182
Campaign Set 2	NIST	NIST SRM 3666	Level II	2	1	Run 2	287536.1	300073.6	0.95822	1122.21722
Campaign Set 2	NIST	NIST SRM 3666	Level II	2	1	Run 3	291387.3	304101.9	0.95819	1122.18336
Campaign Set 2	NIST	NIST SRM 3666	Level II	2	2	Run 1	276909.4	287628.5	0.96273	1134.57252
Campaign Set 2	NIST	NIST SRM 3666	Level II	2	2	Run 2	280980.1	292741.5	0.95982	1131.14361
Campaign Set 2	NIST	NIST SRM 3666	Level II	2	2	Run 3	285692.6	296999.3	0.96193	1133.62664
Campaign Set 2	NIST	NIST SRM 3666	Level II	2	3	Run 1	271309.7	284091.3	0.95501	1131.31181
Campaign Set 2	NIST	NIST SRM 3666	Level II	2	3	Run 2	272056.9	285327.9	0.95349	1129.51093
Campaign Set 2	NIST	NIST SRM 3666	Level II	2	3	Run 3	275922.1	289113.1	0.95437	1130.56009
Campaign Set 2	NIST	NIST SRM 3666	Level III	2	1	Run 1	274576.6	284120.1	0.96641	1183.21323
Campaign Set 2	NIST	NIST SRM 3666	Level III	2	1	Run 2	275232.6	286195.6	0.96169	1177.43888
Campaign Set 2	NIST	NIST SRM 3666	Level III	2	1	Run 3	279523.2	291218.8	0.95984	1175.16786
Campaign Set 2	NIST	NIST SRM 3666	Level III	2	2	Run 1	269884.8	285867.1	0.94409	1171.24268
Campaign Set 2	NIST	NIST SRM 3666	Level III	2	2	Run 2	272046.9	287713.4	0.94555	1173.04948
Campaign Set 2	NIST	NIST SRM 3666	Level III	2	2	Run 3	275068.0	291261.2	0.94440	1171.62890
Campaign Set 2	NIST	NIST SRM 3666	Level III	2	3	Run 1	275333.4	287210.6	0.95865	1182.87110
Campaign Set 2	NIST	NIST SRM 3666	Level III	2	3	Run 2	277700.9	289142.0	0.96043	1185.07298
Campaign Set 2	NIST	NIST SRM 3666	Level III	2	3	Run 3	281381.6	293941.8	0.95727	1181.17252
Campaign Set 2	NIST	NIST SRM 3666	Level IV	2	1	Run 1	276482.0	280110.8	0.98705	1254.73250
Campaign Set 2	NIST	NIST SRM 3666	Level IV	2	1	Run 2	276767.4	281151.7	0.98441	1251.37755
Campaign Set 2	NIST	NIST SRM 3666	Level IV	2	1	Run 3	279942.3	285643.5	0.98004	1245.82867
Campaign Set 2	NIST	NIST SRM 3666	Level IV	2	2	Run 1	280798.1	284959.7	0.98540	1251.00369
Campaign Set 2	NIST	NIST SRM 3666	Level IV	2	2	Run 2	280581.9	285248.5	0.98364	1248.77487
Campaign Set 2	NIST	NIST SRM 3666	Level IV	2	2	Run 3	286572.0	292329.5	0.98030	1244.54035
Campaign Set 2	NIST	NIST SRM 3666	Level IV	2	3	Run 1	280456.8	284135.4	0.98705	1257.28676
Campaign Set 2	NIST	NIST SRM 3666	Level IV	2	3	Run 2	279642.8	285410.8	0.97979	1248.03554
Campaign Set 2	NIST	NIST SRM 3666	Level IV	2	3	Run 3	284001.9	288005.1	0.98610	1256.07271
Campaign Set 2	HSA, Singapore	HRM 3004A-01	Level 1	2	1	Run 1	281210.3	281013.1	1.00070	395.08817
Campaign Set 2	HSA, Singapore	HRM 3004A-01	Level 1	2	1	Run 2	280787.0	280923.8	0.99951	394.61886
Campaign Set 2	HSA, Singapore	HRM 3004A-01	Level 1	2	1	Run 3	285693.2	285668.8	1.00009	394.84484
Campaign Set 2	HSA, Singapore	HRM 3004A-01	Level 1	2	2	Run 1	276600.3	274707.8	1.00689	396.87926
Campaign Set 2	HSA, Singapore	HRM 3004A-01	Level 1	2	2	Run 2	278173.8	276897.3	1.00461	395.98091
Campaign Set 2	HSA, Singapore	HRM 3004A-01	Level 1	2	2	Run 3	280822.6	280768.2	1.00019	394.24018
Campaign Set 2	HSA, Singapore	HRM 3004A-01	Level 1	2	3	Run 1	272935.4	273497.3	0.99795	396.37536
Campaign Set 2	HSA, Singapore	HRM 3004A-01	Level 1	2	3	Run 2	274129.2	273204.2	1.00339	398.53617
Campaign Set 2	HSA, Singapore	HRM 3004A-01	Level 1	2	3	Run 3	277290.3	279428.6	0.99235	394.15192
Campaign Set 2	HSA, Singapore	HRM 3004A-02	Level 2	2	1	Run 1	294400.5	290769.8	1.01249	1197.60251
Campaign Set 2	HSA, Singapore	HRM 3004A-02	Level 2	2	1	Run 2	295945.2	289496.3	1.02228	1209.18217
Campaign Set 2	HSA, Singapore	HRM 3004A-02	Level 2	2	1	Run 3	298878.7	295138.0	1.01267	1197.82476
Campaign Set 2	HSA, Singapore	HRM 3004A-02	Level 2	2	2	Run 1	291795.6	288190.9	1.01251	1202.18310
Campaign Set 2	HSA, Singapore	HRM 3004A-02	Level 2	2	2	Run 2	292652.5	289736.4	1.01006	1199.28201
Campaign Set 2	HSA, Singapore	HRM 3004A-02	Level 2	2	2	Run 3	295019.9	292742.8	1.00778	1196.56758
Campaign Set 2	HSA, Singapore	HRM 3004A-02	Level 2	2	3	Run 1	293200.2	288375.1	1.01673	1207.88749
Campaign Set 2	HSA, Singapore	HRM 3004A-02	Level 2	2	3	Run 2	294697.5	292347.7	1.00804	1197.55853
Campaign Set 2	HSA, Singapore	HRM 3004A-02	Level 2	2	3	Run 3	294056.6	292619.7	1.00491	1193.84336

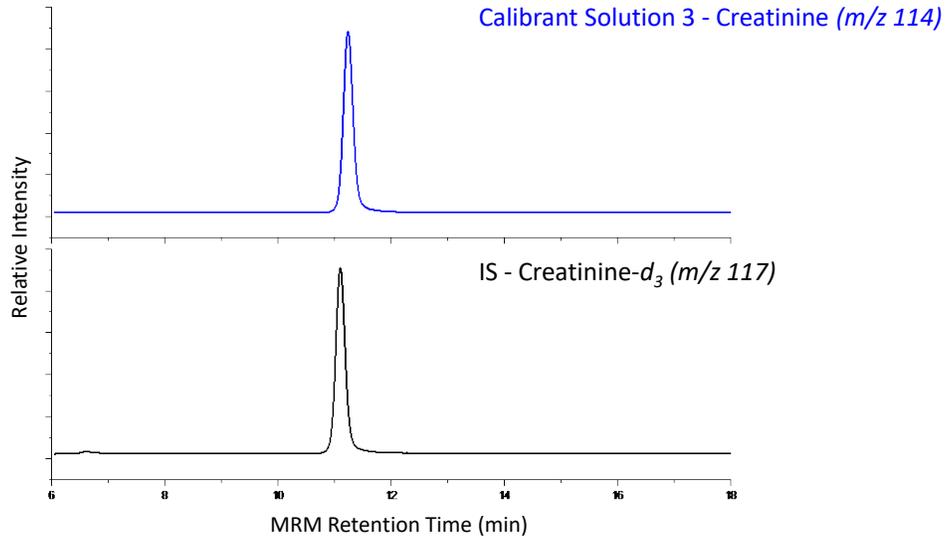


Fig. B1. Representative MRM chromatographic profile of creatinine (m/z 114) and creatinine- d_3 (IS, m/z 117) in Calibration Solution 3 from Prep stock 3 (1:50 fold dilution; refer to Table B3 for creatinine calibration solution). (m/z – mass-to-charge ratio)

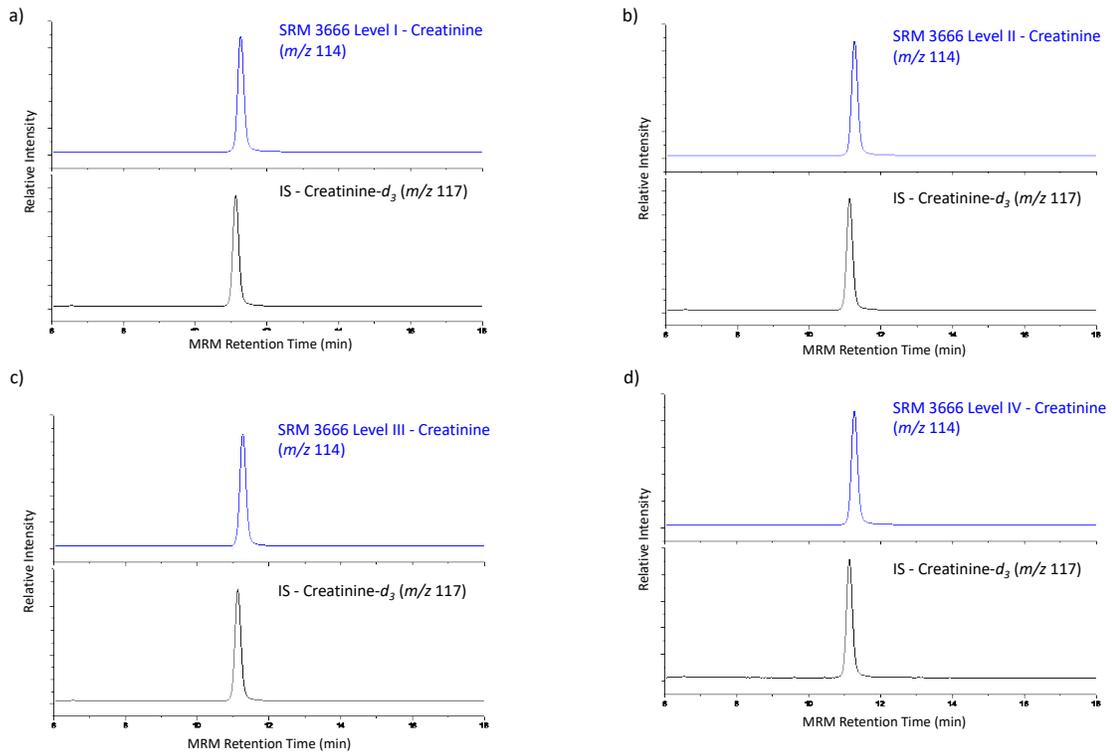


Fig. B2. Representative MRM chromatographic profile of creatinine (m/z 114) and creatinine- d_3 (IS, m/z 117) in NIST SRM 3666 [Level I (a), Level II (b), Level III (c), Level IV (d)]. (m/z – mass-to-charge ratio)

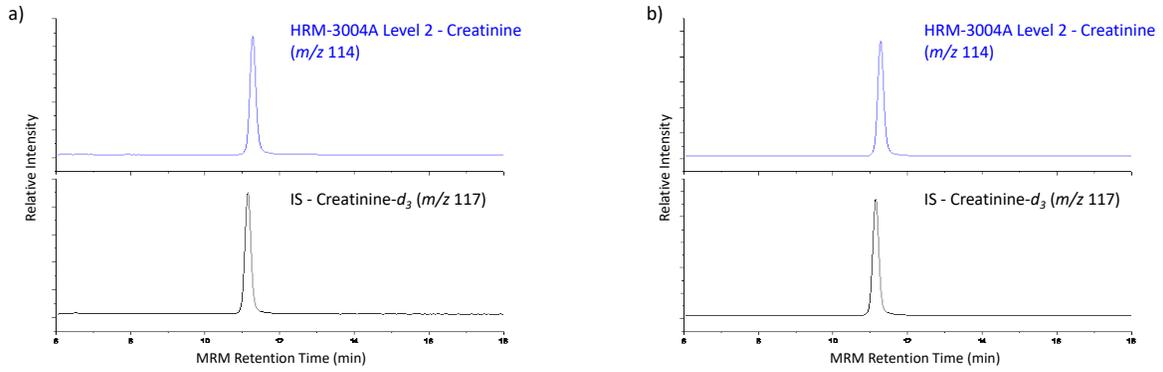


Fig. B3. Representative MRM chromatographic profile of creatinine (m/z 114) and creatinine- d_3 (IS, m/z 117) in HSA HRM-3004A [Level 1(a) and Level 2 (b)]. (m/z – mass-to-charge ratio)

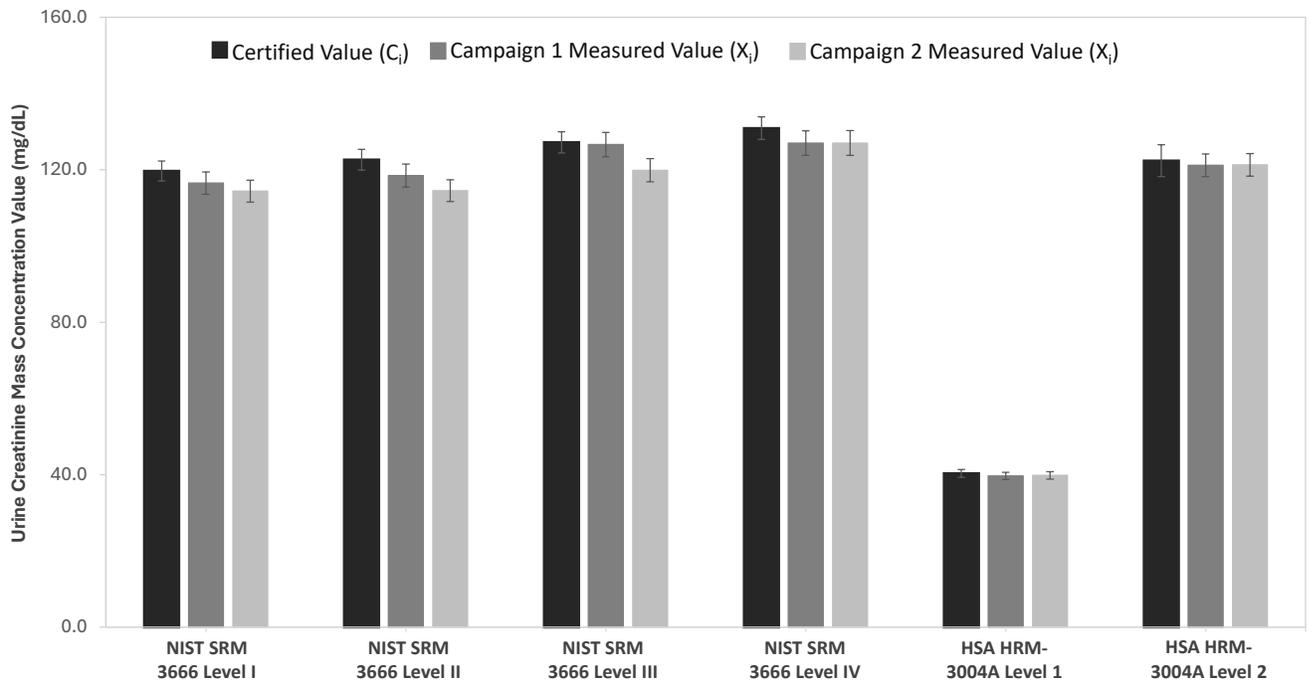


Fig. B4. Plots of certified value versus measured consensus mass concentration (mg/dL) values for urine creatinine for the CRM samples: NIST SRM 3666 Level I (a), NIST SRM 3666 Level II (b), NIST SRM 3666 Level III (b), NIST SRM 3666 Level IV (b), HSA HRM-3004A Level 1 (a), and HSA HRM-3004A Level 2 (b).

The error bars for the certified value represent the expanded uncertainty ($k = 2.201$) of the certified value, and the error bars for the measured values represent the expanded uncertainty ($k = 2.31$) of the mass concentration values ($n = 18$) for each sample.

APPENDIX C – NIST SRM 3666 CERTIFICATE (11 JANUARY 2024)

CRM CERTIFICATE – NIST SRM 3666 (as of 11 January 2024)



Date of Issue:
11 January 2024

Standard Reference Material® 3666 Albumin and Creatinine in Frozen Human Urine

CERTIFICATE OF ANALYSIS

Purpose: The certified values delivered by this Standard Reference Material (SRM) are intended for use in validating measurement procedures and qualifying control materials produced in-house and analyzed using measurement methods for the determination of albumin, creatinine, or the albumin-to-creatinine ratio (ACR) in human urine.

Description: A unit of SRM 3666 consists of four vials, each containing approximately 1.0 mL of frozen pooled human urine. Each vial contains different levels of amount-of-substance of endogenous albumin, creatinine, and ACR (Level I, Level II, Level III, and Level IV).

Certified Values: Certified values are provided in Tables 1 to 4. A NIST certified value is the present best estimate of the true value. Values in Table 1, Table 2 and Table 3 are metrologically traceable to the International System of Units (SI) for mass fraction and mass concentration [1]. Values in Table 4 are metrologically traceable to the SI units of mass and length [1].

The certified mass fraction and mass concentration values with associated uncertainties for albumin provided in Table 1 are based on the results obtained from the analytical results determined using the NIST isotope dilution-liquid chromatography-tandem mass spectrometry (ID-LC-MS/MS) candidate reference measurement procedure for albumin in urine [2,3].

Table 1. Certified Values for Albumin in SRM 3666

Material Level	Mass Fraction ^(a) (mg/g)	Mass Concentration ^(a,b) (mg/L)
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 $U(x) = k u_c$, where u_c is the combined uncertainty, and k is the coverage factor. For calculation of the combined uncertainty for albumin certified values see Reference 3. For the certified values shown in Table 1, $k = 2$. 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 4.

Period of Validity: The certified values delivered by SRM 3666 are valid within the measurement uncertainty specified until 10 May 2028, provided the SRM is handled and stored in accordance with instructions given in this certificate. The certified values are nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

Maintenance of Certified Values: NIST will monitor this SRM over the period of its validity. If substantive technical changes occur that affect the certification, NIST will issue an amended certificate through the NIST SRM website (<https://www.nist.gov/srm>) and notify registered users. SRM users can register online from a link available on the NIST SRM website or fill out the user registration form that is supplied with the SRM. Registration will facilitate notification. Before making use of any of the values delivered by this material, users should verify they have the most recent version of this documentation, available through the NIST SRM website (<https://www.nist.gov/srm>).

Michael Tarlov, Chief
Biomolecular Measurement Division
Certificate Revision History on Last Page

Steven J. Choquette, Director
Office of Reference Materials

The certified mass fraction and mass concentration values with associated uncertainties for creatinine provided in Table 2 are based on the results obtained from the analytical results determined using a modification of the NIST isotope dilution-liquid chromatography-mass spectrometry (ID-LC-MS) reference measurement procedure for creatinine in serum [4].

Table 2. Certified Values for Creatinine in SRM 3666

Material Level	Mass Fraction ^(a) ($\mu\text{g/g}$)	Mass Concentration ^(a,b) (mg/dL)
Level I	1178.96 \pm 25.05	119.69 \pm 2.63
Level II	1209.58 \pm 26.28	122.65 \pm 2.73
Level III	1249.58 \pm 26.37	127.19 \pm 2.78
Level IV	1289.01 \pm 27.71	130.90 \pm 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 $U(x) = k u_c$, 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 4.

Table 3. Certified Values for ACR in SRM 3666

Material Level	Mass Fraction ^(a,b) (mg/g)
Level I	6.92 \pm 0.95
Level II	25.36 \pm 2.22
Level III	88.66 \pm 8.70
Level IV	275.40 \pm 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 $U(x) = k u_c$, where u_c is the combined uncertainty, and k is the coverage factor. For the certified values shown in Table 3, $k = 2$. 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 ACR is based on the combination of the certified values for albumin (Table 1) and creatinine (Table 2).

Table 4. Certified Values for Human Urine Density of SRM 3666 at 19 °C

Material Level	Density ^(a) (g/mL)
Level I	1.01519 \pm 0.00058
Level II	1.01397 \pm 0.00048
Level III	1.01784 \pm 0.00058
Level IV	1.01549 \pm 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 $U(x) = k u_c$, where u_c is the combined uncertainty, and k is the coverage factor. For the certified values shown in Table 4, $k = 2$. 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 $U(x)/2$.

Safety: SRM 3666 IS INTENDED FOR RESEARCH USE. This is a human-source material. Handle product as a biohazardous material capable of transmitting infectious disease. The supplier has reported that each donor unit of urine used in the preparation of this product was tested by FDA-licensed tests and found to be non-reactive for human immunodeficiency virus (HIV), HIV-1 and HIV-2, hepatitis B surface antigen, and hepatitis C. However, no known test method can offer complete assurance that hepatitis B virus, hepatitis C virus, HIV, or other infectious agents are absent from this material. As such, this human urine-based product should be handled at Biosafety Level 2 as recommended by the Centers for Disease Control and Prevention's Biosafety in Microbiological and Biomedical Laboratories (6th edition) [5] for human-derived urine products where the presence of an infectious agent may be unknown.

Source: This SRM was developed after an appropriate human subjects research determination.

Storage: SRM 3666 is shipped frozen (on dry ice) and, upon receipt, should be stored frozen until ready for use. A freezer temperature of $-20\text{ }^{\circ}\text{C}$ is acceptable for storage for up to one week. If a longer storage time is anticipated, the material should be stored at or below $-70\text{ }^{\circ}\text{C}$. The SRM should not be exposed to sunlight or ultraviolet radiation.

Use: SRM 3666 is provided as a set of four vials of frozen human urine. The vial (or vials) to be used should be allowed to thaw at room temperature for at least 30 min. The contents of the vial should then be gently mixed prior to removal of a test portion for analysis. The certification only applies to the initial use and the same results are not guaranteed if the remaining material is used at a later date.

Additional Information: Full details on the production, analysis, and statistical evaluation of SRM 3666 are provided in reference 6.

REFERENCES

- [1] Beauchamp, C.R.; Camara, J.E.; Carney, J.; Choquette, S.J.; Cole, K.D.; DeRose, P.C.; Duewer, D.L.; Epstein, M.S.; Kline, M.C.; Lippa, K.A.; Lucon, E.; Molloy, J.; Nelson, M.A.; Phinney, K.W.; Polakoski, M.; Possolo, A.; Sander, L.C.; Schiel, J.E.; Sharpless, K.E.; Toman, B.; Winchester, M.R.; Windover, D.; *Metrological Tools for the Reference Materials and Reference Instruments of the NIST Material Measurement Laboratory*; NIST Special Publication 260-136, 2021 edition; National Institute of Standards and Technology, Gaithersburg, MD (2021); available at <https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-136-2021.pdf> (accessed Jan 2024).
- [2] Beasley-Green, A.; Burris, N.; Bunk, D.M.; Phinney, K.W.; *Multiplexed LC-MS/MS Assay for Urine Albumin*; *J. Proteome Res.*, Vol. 13, pp. 3930–3939 (2014).
- [3] Beasley-Green, A.; Heckert, A. *Estimation of Measurement Uncertainty for the Quantification of Protein by ID-LC-MS/MS*; *Anal. Bioanal. Chem.*, Vol. 415, pp. 3265-3274 (2023).
- [4] Dodder, NG; Tai, S.S.C.; Sniegoski, L.T.; Zhang, N.F.; Welch, M.J. *Certification of Creatinine in a Human Serum Reference Material by GC-MS and LC-MS*; *Clin. Chem.*, Vol. 53, pp. 1694–1699 (2007).
- [5] CDC/NIH; *Biosafety in Microbiological and Biomedical Laboratories, 6th ed.*; Meehan, P.J.; Potts, J., Eds.; U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention and National Institutes of Health; National Institutes of Health; HHS Publication (CDC) 300859 (2020); available at <https://www.cdc.gov/labs/BMBL.html> (accessed Jan 2024).
- [6] Beasley-Green, A.; Camara, J.; Heckert, N.A. *Certification of Standard Reference Material[®] 3666 Albumin and Creatinine in Frozen Human Urine*; NIST Special Publication 260-238-upd1; National Institute of Standards and Technology, Gaithersburg, MD (2023); available at <https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-238-upd1.pdf> (accessed Jan 2024).

If you use this SRM in published work, please reference:

Beasley-Green A, Camara J, Heckert NA (2023) Certification of Standard Reference Material[®] 3666 Albumin and Creatinine in Frozen Human Urine. (National Institute of Standards and Technology, Gaithersburg, MD), NIST Special Publication (SP) 260-238-upd1. <https://doi.org/10.6028/NIST.SP.260-238-upd1>

Certificate Revision History: 11 January 2024 (Updated SP 260-238 title; editorial changes); 28 June 2023 (Original certificate date).

Certain commercial equipment, instruments, or materials may be identified in this Certificate of Analysis to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the Office of Reference Materials 100 Bureau Drive, Stop 2300, Gaithersburg, MD 20899-2300; telephone (301) 975-2200; e-mail srminfo@nist.gov; or the Internet at <https://www.nist.gov/srm>.

* * * * * End of Certificate of Analysis * * * * *

APPENDIX D – HSA HRM-3004A CERTIFICATE (15 APRIL 2025)

CRM CERTIFICATE – HSA HRM-3004A (as of 15 April 2025)

Chemical Metrology Division
Applied Sciences Group
Health Sciences Authority
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Website: www.hsa.gov.sg
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Ref. No.: CML-HRM-J3004A/09
Date of Issue: 15 Apr 2025

Certificate of Analysis

CERTIFIED REFERENCE MATERIAL
HRM-3004A

Albumin and Creatinine in Human Urine

Batch Number

STY-0018-053
STY-0018-054

Foreword

A unit of the certified reference material (CRM) HRM-3004A consists of two vials of frozen human urine sample with different albumin and creatinine concentration levels. Each vial contains 2 ml of frozen human urine. The urine materials appear as a light yellow or yellow liquid after thawing.

The CRM was produced with reference to the requirements set out in ISO/IEC 17025:2017 [1], ISO 17034:2016 [2] and ISO Guide 35:2017 [3].

Certified Concentration Values

The certified concentration values for albumin and creatinine in HRM-3004A are provided in Table 1 and Table 2, respectively.

The certified values for albumin with the unit of mg/L in Table 1 were calculated from the concentration value of µg/g, the measured urine density at 23 °C (1.0068 g/ml and 1.0167 g/ml for STY-0018-053 and STY-0018-054, respectively). The certified values for creatinine with the unit of mmol/L in Table 2 were calculated from the concentration value of mg/kg, the measured urine density at 23 °C, and the relative molecular mass of creatinine (113.12 g/mol).

Table 1. Certified Values of Albumin in HRM-3004A

Analyte	STY-0018-053	STY-0018-054
	(mg/L)	(mg/L)
Albumin	40.1 ± 2.4	226 ± 11

Table 2. Certified Values of Creatinine in HRM-3004A

Analyte	STY-0018-053	STY-0018-054
	(mmol/L)	(mmol/L)
Creatinine	3.567 ± 0.093	10.82 ± 0.37

Each certified value is the mean of measurements of at least six samples taken from a minimum of three vials. The certified concentration values for HRM-3004A were determined using isotope dilution mass spectrometry (IDMS). A four-point calibration curve was used in the measurements.

The associated measurement uncertainty of each certified value was evaluated in accordance with ISO/IEC Guide 98-3:2008 [4]. The expanded uncertainty (coverage factor of 2) corresponded to a level of confidence of about 95%.

Validity

The certified values of HRM-3004A are valid within the specified measurement uncertainty until 15 Apr 2027. The validity of HRM-3004A will be extended if it is tested to be sufficiently stable for continuous use. The certified values of HRM-3004A are invalid when the urine material has deteriorated or is mishandled.

Source of Materials

The urine materials were prepared by Solomon Park Research Laboratories (Kirkland, WA, USA).

Commutability

Commutability studies of albumin and creatinine in HRM-3004A were conducted by analysing 25 to 30 patient urine samples and the CRM by both liquid chromatography-isotope dilution tandem mass spectrometric (LC-IDMS/MS) method and routine methods (immunoturbidimetric method for albumin and Jaffe and enzymatic methods for creatinine) using various clinical analysers, namely Roche Cobas c702, Roche Cobas c311, Siemens Atellica Solution CH, Beckman AU5800, and Abbott Architect ci16200. The results demonstrated satisfactory commutability of albumin and creatinine in HRM-3004A for these clinical analysers, which suggested that HRM-3004A is commutable for these clinical analysers.

Homogeneity

Homogeneity testing on albumin and creatinine was performed on two subsamples taken from eleven vials using a Roche Cobas C501 chemistry analyzer. The sample size taken for homogeneity testing was 6 μL for albumin and 10 μL for creatinine. No significant differences in the between and within-vial variances were found using F-test (ANOVA) at 95 % confidence level. The u_{inh} was evaluated from the uncertainty due to between-vial inhomogeneity.

Stability

The stability of albumin and creatinine in HRM-3004A stored at a temperature of below $-60\text{ }^{\circ}\text{C}$ was evaluated on at least three occasions over a period of up to three months. The results showed that the analytes were stable when stored at below $-60\text{ }^{\circ}\text{C}$ over the study period [3]. The u_{stab} was evaluated from the standard error of the slope.

Analytical Methods

For the determination of albumin, a fully validated LC-IDMS/MS method was used [5]. The method involved spiking with isotope-labeled albumin, proteolysis of albumin using trypsin, separation using an Agilent Zorbax Eclipse Plus C18 column, followed by tandem mass spectrometric measurement of the signature peptides of albumin.

For the determination of creatinine, a fully validated LC-IDMS/MS method modified from previously published method [6] was used. The method involved spiking with isotope-labeled creatinine, separation using an Agilent Zorbax SB-Aq column, followed by tandem mass spectrometric measurement.

Metrological Traceability

The certified concentration values are traceable to the International System of Units (SI) through the use of albumin CRM 6202a from the National Metrology Institute of Japan (NMIJ), and creatinine

CRM (SRM 914a) from the National Institute of Standards and Technology (NIST).

Intended Use

HRM-3004A is a secondary measurement standard and is intended for use in the validation of methods or as quality control material for the determination of albumin and creatinine in human urine. Users may refer to ISO 33403:2024 [7] for the recommended statistical treatment of the certified reference value and the associated uncertainty of the CRM as control materials.

Warning and Safety Precautions for Users

HRM-3004A is intended for in-vitro use only and shall be handled as a biohazardous material with the potential of transmitting infectious diseases. Hence, this material shall be handled using biosafety level 2 (or higher) practices, equipment, and facility [8].

Instructions for Use

HRM-3004A should be treated the same as patient specimens. Accordingly, these materials should be handled and disposed according to associated regional, national and local legislation and regulations for any potentially infectious human specimen.

Prior to use, the CRM should be thawed at room temperature (between 18 °C to 25 °C), then analysed immediately. The materials should be mixed well by gentle swirling before withdrawing any aliquots. The certified values may not be valid for re-thawed and opened bottles as the stability of both albumin and creatinine subjected to such conditions has not been investigated.

The recommended minimum sample size of HRM-3004A is 6 µL for albumin and 10 µL for creatinine. The certified values may not be valid if smaller amounts are taken.

Transport and Storage

The CRM is transported in frozen state (in dry ice). Upon receipt, it should be stored at below – 60 °C. The CRM should not be exposed to sunlight or ultraviolet radiation. Storage of the thawed material at room temperature or in the refrigerator may result in changes in the certified values.

Further Information

Please direct all enquiries regarding this CRM to the contact in this Certificate.

References

- [1] ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories.
- [2] ISO 17034:2016 General requirements for the competence of reference material producers.
- [3] ISO Guide 35:2017 Reference materials – Guidance for characterisation and assessment for homogeneity and stability.
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- [6] Dodder, N. G.; Tal, S.; Sniegowski, L. T.; Zhang, N. F.; Welch, M. J.; *Clin. Chem.*, 2007, 53, 1694-1699.
- [7] ISO 33403:2024 Reference materials – Requirements and recommendations for use.
- [8] U.S Department of Health and Human Services; *Biosafety in Microbiological and Biomedical Laboratories*, 5th ed.; HHS Publication No. (CDC) 21-1112.

Certificate Revision Records

Certificate Ref. No.	Date of issue	Reason for issuance
CML-HRM-3004A/01	06 Feb 2018	Issuance of first certificate
CML-HRM-3004A/02	12 Nov 2018	Extension of expiry date
CML-HRM-3004A/03	30 Jan 2019	Extension of expiry date
CML-HRM-3004A/04	06 Jan 2020	Extension of expiry date
CML-HRM-3004A/05	20 Jan 2021	Extension of expiry date
CML-HRM-3004A/06	24 Jan 2022	Extension of expiry date
CML-HRM-3004A/07	25 May 2023	Extension of expiry date
CML-HRM-3004A/08	18 Nov 2024	Addition of information on commutability
CML-HRM-3004A/09	15 Apr 2025	Extension of expiry date

Note

HSA does not assume any liability with respect to any loss caused by improper use and/or storage of the CRM by the customer.



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Division Director
Chemical Metrology Laboratory
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