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A study examining the bias of albumin and albumin/creatinine ratio measurements in urine

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Abstract

Background: The objective of the study was to examine the bias of albumin and albumin/creatinine (ACR) measurements in urine.
Methods: Pools of normal human urine were augmented with purified human serum albumin to generate a series of 12 samples covering the clinical range of interest for the measurement of ACR. Albumin and creatinine concentrations in these samples were analyzed three times on each of 3 days by 24 accredited laboratories in Canada and the USA. Reference values (RV) for albumin measurements were assigned by a liquid chromatography-tandem mass spectrometry (LC-MS/MS) comparative method and gravimetrically. Ten random urine samples (check samples) were analyzed as singlets and albumin and ACR values reported according to the routine practices of each laboratory.
Results: Augmented urine pools were shown to be commutable. Gravimetrically assigned target values were corrected for the presence of endogenous albumin using the LC-MS/MS comparative method. There was excellent agreement between the RVs as assigned by these two methods. All laboratory medians demonstrated a negative bias for the measurement of albumin in urine over the concentration range examined. The magnitude of this bias tended to decrease with increasing albumin concentrations. At baseline, only 10% of the patient ACR values met a performance limit of RV ±15%. This increased to 84% and 86% following post-analytical correction for albumin and creatinine calibration bias, respectively.
Conclusions: International organizations should take a leading role in the standardization of albumin measurements in urine. In the interim, accuracy based urine quality control samples may be used by clinical laboratories for monitoring the accuracy of their urinary albumin measurements.

Keywords: albuminuria; albumin/creatinine ratio (ACR); measurements.

Introduction

The measurement of urinary albumin and the reporting of albumin/creatinine ratio (ACR) play an important role in the diagnosis and management of kidney disease. The recent KDIGO guidelines recommend that ACR be used in conjunction with estimated glomerular filtration rate (eGFR) in the clinical stratification of patients with kidney disease [1]. It is noteworthy that these guidelines recommend that the historical term for the measurement of albumin in urine ("micro albuminuria") should no longer be used.

There are many unanswered questions with respect to the accuracy of albumin and ACR measurements in urine. These issues have been summarized previously [2]. Foremost among these is albumin and creatinine measurements in urine have not been standardized and as such significant inter-method differences in reported ACR values have been observed [3]. As of yet there are no credentialed reference methods for the measurement of these analytes in urine. A candidate reference method for the measurement of urinary albumin is currently under development [4].

The present study examined the accuracy and precision of albumin and creatinine measurements in urine as provided by 24 accredited clinical laboratories in Canada and the USA.

Materials and methods

Pools of urine were collected from two healthy disease-free male subjects. Single donor Pool A had a relatively low creatinine concentration whereas single donor Pool B had a higher creatinine concentration.

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Both pools were augmented gravimetrically with purified human serum albumin as supplied by SCRIPPS Laboratories, San Diego, CA, USA. The albumin was used as supplied without further characterization. The purity of this material was checked and adjusted to 100% on the basis of reference values as assigned by a credentialed reference method for the measurement of total protein [5, 6]. The combined expanded uncertainty for this reference method as operated in the Canadian External Quality Assessment Laboratory (CEQAL) reference method laboratory is 1.2% with a coverage factor of 2. The coverage factor provides a particular confidence level to the expanded uncertainty. The performance of this method is such that the assigned value is estimated to be within 1.2% of the true value with a 95% level of confidence. This material was subsequently used gravimetrically to produce albumin stock solutions that were prepared in the appropriate urine A and B pool matrices.

The creatinine concentration in each of the pools was held constant throughout the augmentation process. Unlike serum, the measurement of creatinine in urine has not been standardized. In the present study, the median of all submitted creatinine test results for urine A and B was taken as the reference value for the measurement of this analyte in the pool samples under consideration. These median values were subsequently used to recalculate laboratory specific creatinine corrected ACR values.

The pools were augmented so as to produce six samples within each pool (A1-6, B1-6) with ACR values <3, 3–29, >30 mg/mmol [1]. Very high albumin concentrations were not examined.

All of the participating laboratories received common sets of the Pool A and B samples together with 10 patient check samples. The check samples were from single donor subjects and were selected from residual urines that had been submitted for routine clinical reporting of ACR. The patient check samples had liquid chromatography-tandem mass spectrometry (LC-MS/MS) albumin concentrations ranging from 9.9 to 1261 mg/L. All pool and check samples were sterilized by filtration (0.2 µm) prior to shipping on gel pack by overnight courier to the participating laboratories.

LC-MS/MS values were assigned to all of the samples by a comparative method for the measurement of urinary albumin [4]. The mean value obtained from two separate sample digestions followed by duplicate analyses for each digest (n=4 test results) was taken as the assigned reference value for a given sample.

A total of 24 accredited medical laboratories in USA were recruited to participate in the study. A cross section of manufacturer's instrumentation models and reagent systems were included (Abbott, Siemens, Beckman, Ortho and Roche). The specific details on instrument platforms were not collected. All of the analytical methods for the measurement of urinary albumin were immunological.

Each laboratory was asked to measure albumin and creatinine in the A and B pool samples three times on each of 3 days. The median of the nine results was taken as the laboratories' reported value for that sample. The within-sample between-day precision for the laboratory's method was calculated from these data. In the case of the patient check samples, the laboratories were asked to analyze them as singlets and to report the albumin and ACR values according to the routine practices of their laboratory.

Results

Of the 12 pools and 10 patient check samples only one, pool B-4, demonstrated an all laboratory median positive bias to LC MS/MS. All of the 24 laboratories demonstrated a positive bias to LC MS/MS on this sample. The LC MS/MS analysis was not repeated. Pool B-4 was deemed to be an outlier and was removed from the study data set. The gravimetric albumin assigned values were compared to the LC-MS/MS values for the pool A+B samples. The concentration of albumin in the non-augmented base pools (A+B) was determined from the linear regression intercepts that were obtained from these data.

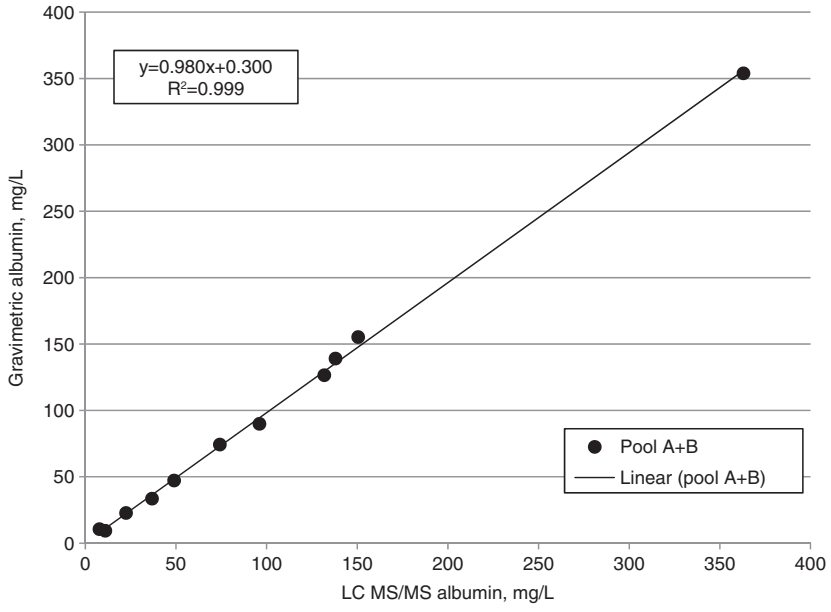

Figure 1: Gravimetric assigned albumin values vs. LC MS/MS values for pools A+B.

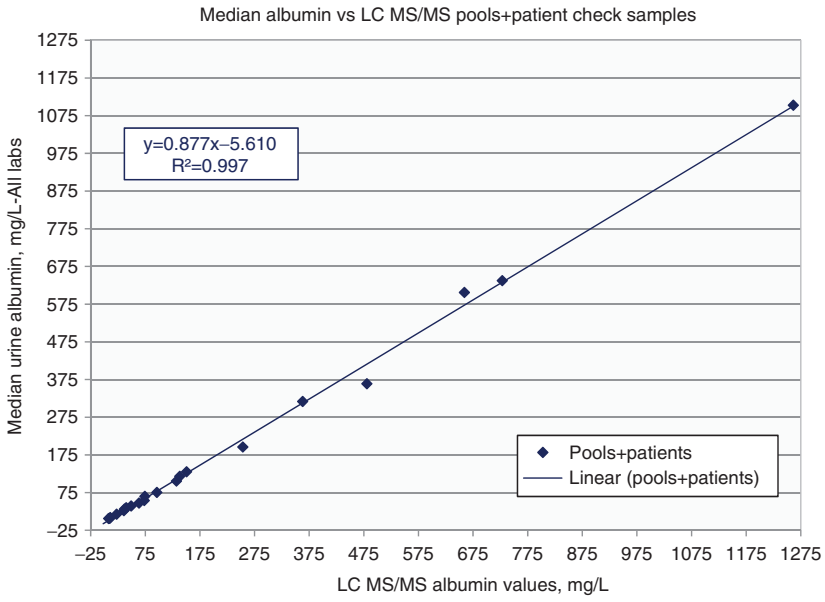

Figure 2: Median albumin values for pools A+B and patient check samples vs. LC-MS/MS values for urinary albumin.

The gravimetrically assigned albumin values were adjusted for the presence of endogenous albumin in the pools on the basis of these intercepts. The adjusted gravimetric assigned values are compared to the LC-MS/MS values in Figure 1.

It is well recognized in serum that sample matrix can have a significant impact on method performance. A recent report emphasizes the importance of matrix issues and the need for confirming the commutability of accuracy-based samples that are used for the standardization of albumin measurements in urine [2].

In Figure 2, the median patient check sample data have been combined with the pool A+B data and plotted against the LC MS/MS assigned values for albumin. The correlation by linear regression had an r^2 of 0.997, a slope of 0.87 and an intercept of –5.6 mg/L. In this study there was no significant difference between the pools and patient check sample data, an observation which attests to the commutability of the pools.

The within-sample, between-day median precision data for Pools A+B are presented in Tables 1 and 2. The between laboratory minimum/maximum range for reported %CVs in Table 1 suggests that precision is a significant problem for some methods or laboratories. Table 2 compares the all laboratory median precision data from the patient check samples with the all laboratory median precision data from Pools A+B at comparable concentrations of albumin. There is very good agreement between these CVs adding further evidence to the commutability of the Pool A+B samples.

The all laboratory median bias across all samples was negative relative to the comparative reference value. The magnitude of this bias tended to decrease as the concentration of albumin in the samples increased (see Table 3).

Patient check samples 2, 6, 7 and 8 had ACR values ranging from 18 mg/mmol (category A2 – moderately increased) to 38 mg/mmol (category A3 – severely increased) [1]. The albumin concentration in these samples were deemed to be important for the staging of kidney disease and selected for further analysis. The laboratory specific performance data from Pool A+B were used in generating regression equations for each laboratory

Table 1: The min/max median %CV (all laboratories) for the measurement of albumin in pools A and B ordered according to increasing pool albumin concentration.

Sample ID	LC MS/MS, mg/L	All laboratories	
		Min %CV	Max %CV
A-6	7.81	0	21.5
B-6	11.06	1.5	16.3
A-5	22.51	1.0	12.6
B-5	36.85	1.1	11.8
A-4	49.00	0.7	8.3
B-3	74.21	0.7	8.9
A-3	96.08	0.5	9.8
A-1	131.88	0.7	13.4
B-2	138.00	0.4	10.1
A-2	150.50	0.6	9.1
B-1	363.00	0.7	4.0

Table 2: A comparison of median precision data at comparable median urinary albumin concentrations for patient check samples and pool samples.

Patient check sample	All laboratory median albumin concentration, mg/L	Pool sample	All laboratory median albumin concentration, mg/L	Patient check sample all laboratory median, %CV	Pool sample all laboratory median, %CV
1	6.7	A-6	6.4	30.1	24.1
3	27.0	B-5	31.4	11.8	11.3
4	54.0	B-3	65.2	8.9	9.7
5	34.8	A-4	39.6	10.1	10.4
7	363.8	B-4	385.9	6.5	6.3

Table 3: The median % bias to reference for the measurement of albumin in urine with low, medium and high concentration dependent stratification.

	Albumin, mg/L		Hi/low %bias to reference (all laboratories)	Median %bias to reference (all laboratories)	Stratified albumin concentration	Mean stratified %bias to reference
	LC MS/MS reference	All laboratories median				
A-6	7.8	6.4	1.1/−61.6	−18.1	Albumin <100 mg/L	−19.6
B-6	11.1	8.9	8.5/−63.8	−19.5		
A-5	22.5	17.7	−2.3/−51.1	−21.4		
Check 3	35.8	27.0	−5.6/−46.9	−24.6		
B-5	36.9	31.4	3.7/−36.5	−14.8		
Check 5	40.0	34.8	5.2/−35.1	−13.1		
A-4	49.0	39.6	−6.1/−36.7	−19.2		
Check 6	63.4	47.3	−10.2/−38.5	−25.4		
Check 4	73.0	54.0	−7.4/−37.0	−26.0		
B-3	74.2	65.2	5.5/−20.5	−12.1		
A-3	96.1	75.4	−7.8/−30.3	−21.5		
B-2	128.5	118.3	12.1/−15.2	−7.9	Albumin <300 mg/L	−16.0
A-1	131.9	105.5	−4.5/−28.3	−20.0		
A-2	150.5	130.3	3.0/−21.6	−13.4		
Check 2	253.4	196.0	−1.3/−27.2	−22.6		
B-1	334.8	316.8	3.1/−16.7	−5.4	Albumin >300 mg/L	−12.6
Check 7	480.5	363.8	−11.6/−31.8	−24.3		
Check 8	659.0	606.1	5.3/−19.6	−8.0		
Check 10	728.5	637.3	5.2/−25.0	−12.5		

which were subsequently used for post-analytical correction of their method's albumin calibration bias.

The impact of eliminating calibration biases for the measurement of urinary albumin and creatinine on ACR values is presented in Table 4 and Figures 3–5. The performance limits (designated as boxes within the figures) are ±10%, ±15% and ±20% relative to the ACR reference value. These performance limits were set arbitrarily.

The correction of albumin calibration bias reduced the magnitude of the between laboratory variation (see Table 4 change in %CV). Correcting calibration bias for creatinine in these laboratories further reduced the network wide CV but not to the same extent as was seen with albumin.

At baseline, 10% of the reported ACR values for patient check samples 2, 6, 7 and 8 met the ±15% performance limit. Following correction for albumin calibration bias, 84% of the laboratories were able to meet the 15% performance limit. This increased to 86% with the correction of creatinine calibration bias.

Discussion

In this study there was a good correlation between the gravimetrically assigned target values for albumin and the values as assigned by the LC-MS/MS comparative method.

Table 4: Impact of post-analytical correction of calibration bias for albumin (Alb) and creatinine (Cr) measurements in urine on reported ACR values (mg/mmol) for patient check samples 2, 6, 7 and 8.

		Mean	SD	%CV
Check 6	Baseline ACR	14.6	1.37	9.37
	Alb corrected ACR	17.7	1.29	7.27
	Alb+Cr corrected ACR	18.5	1.09	5.90
Check 2	Baseline ACR	19.5	1.44	7.39
	Alb corrected ACR	22.9	0.98	4.30
	Alb+Cr corrected ACR	23.9	0.81	3.38
Check 7	ACR	26.1	1.92	7.34
	Alb corrected ACR	30.2	1.36	4.50
	Alb+Cr corrected ACR	31.5	1.32	4.17
Check 8	ACR	32.2	2.54	7.87
	Alb corrected ACR	37.2	2.42	6.49
	Alb+Cr corrected ACR	38.8	2.33	6.01

The combined accuracy and precision performance data from Pools A+B and the patient check samples support the conclusion that the gravimetrically prepared samples in Pools A and B are commutable and as such could be used to confirm the accuracy of albumin measurements in urine. Post-analytical correction for calibration bias has been used previously as an interim strategy for the standardization of serum creatinine testing until such time as instrument manufacturers were able to establish IDMS traceability for their creatinine calibrations [7]. A similar approach could be used as an interim strategy for the standardization of albumin measurements in urine pending the success of international efforts directed towards the standardization of this analyte in urine.

With patient check samples and pools, the median measurements of albumin in urine were negatively biased to the LC-MS/MS value (average across all samples –16.1%). The magnitude of this negative bias decreased as the concentration of albumin in the samples increased. Although the all laboratory median bias was negative and tended to decrease as the concentration of albumin increased, it is evident from the all laboratory high/low percent bias to reference data in Table 3 that between laboratory biases can vary considerably, with some laboratories reporting a positive bias on a given sample whereas others are reporting a negative bias. This variability in bias is most pronounced in samples having the lowest concentrations of albumin (A6, B6, A5, and Check 3). It is noteworthy that the accurate measurement of albumin at these concentrations is of paramount importance for the clinical diagnosis and management of kidney disease.

The study by Bachmann et al. [3] with the testing of clinical samples by instrument manufacturers observed a

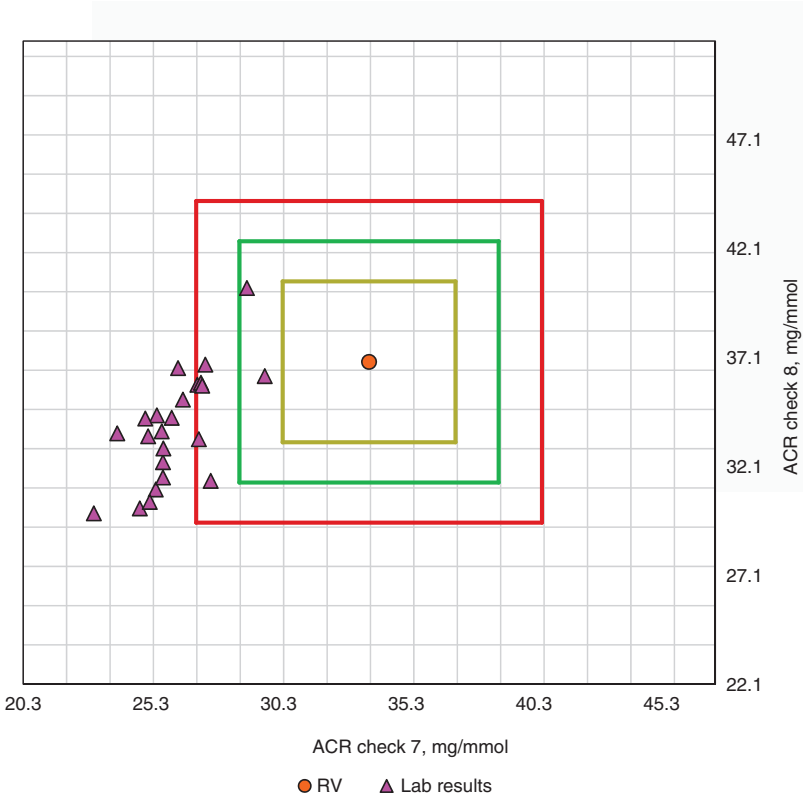

Figure 3: Calculated urinary ACR values for patient check samples 7 and 8. Total error boxes: 10%, 15%, 20%.

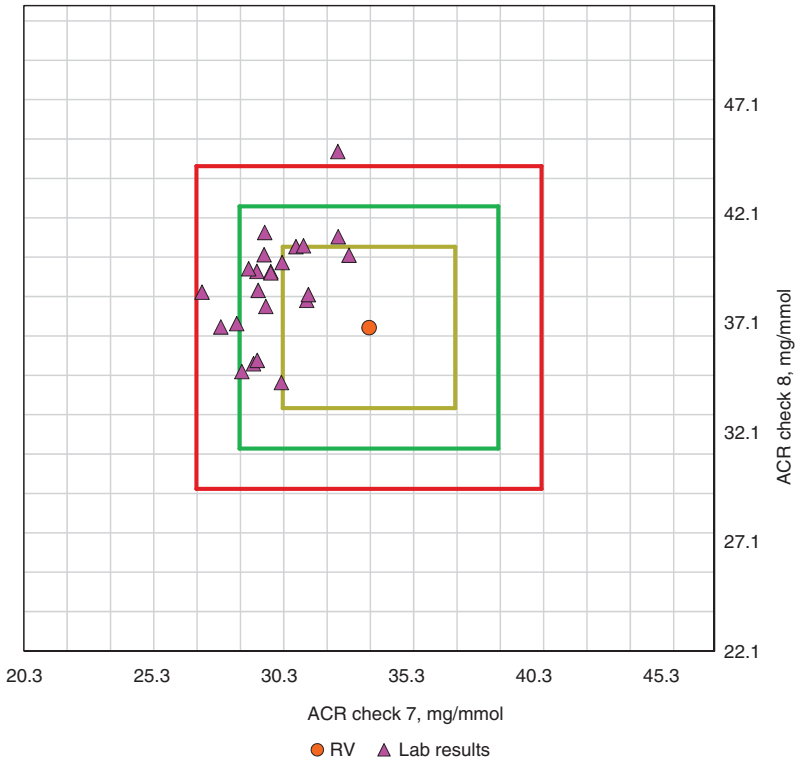

Figure 4: Calculated urinary ACR values for patient check samples 7 and 8 following correction for albumin calibration bias. Total error boxes: 10%, 15%, 20%.

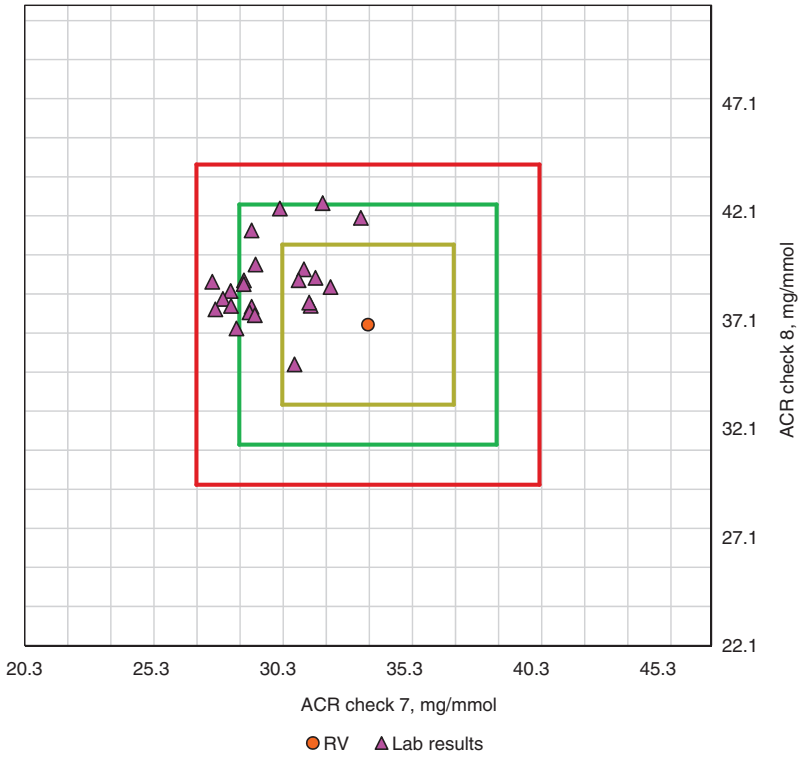

Figure 5: Calculated urinary ACR values for patient check samples 7 and 8 following correction for albumin and creatinine calibration bias. Total error boxes: 10%, 15%, 20%.

positive bias with some methods whereas other methods were reporting a negative bias on the same sample. This variability in bias may reflect differences in antibody specificity for the measurement of albumin and albumin fragments in these samples by these methods. The current study examined the testing of urinary albumin as provided by medical laboratories in Canada and the USA and utilized urine from two healthy donors together with an albumin augmentation process that held the matrix constant. Under these conditions the relative contribution of albumin fragments to the reported value for albumin would have been held constant across the samples. This provided a data set for assessing the accuracy of urinary albumin measurements alone without the confounding influence that variability in the concentration of albumin fragments might be having on the reported results.

The serum protein reference material ERM-DA470k/IFCC [8] is used by many instrument manufacturers for calibrating the measurement of albumin in urine (personal communications). It is noteworthy that the reference value for albumin in this material has been assigned by immunological methods. The observed negative median bias in the current study may reflect matrix issues that manifest when this human serum reference material is diluted in urine.

Conclusions

The laboratory to laboratory variability in urinary albumin measurements is significant calling into question the validity of applying universal ACR cut points in clinical decision-making [1]. The minimum/maximum precision data from this study indicate that some methods and/or laboratories have unacceptably high levels of imprecision. On aggregate the performance data from this group of laboratories had a negative median% bias relative to the LC-MS/MS comparative method and gravimetrically assigned reference values. The magnitude of this bias decreased as the concentration of albumin in the sample increased. Assuming optimal precision, it is evident that the largest improvement in ACR reporting would be achieved by eliminating albumin calibration bias. There is a need for the standardization of albumin measurements in urine and the optimization of methods for the accurate quantitation of urinary albumin at the lower concentrations that are needed for clinical diagnosis and management of kidney disease.

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