COLLEGE of AMERICAN PATHOLOGISTS

Educational Discussion: Urine Albumin, Total Protein, and Creatinine

2018-A Accuracy-Based Urine (ABU)

Performance of urine albumin, total protein and creatinine from the U-A 2018 and ABU-A 2018 Surveys

The U-A 2018 Survey included a fresh-frozen pooled urine sample from donors who had elevated urine albumin. Samples U-03 and U-06 were the same fresh-frozen pooled urine material with different analytes measured in each sample. The urine was kept cold during collection and storage, pooled, filtered and frozen in aliquots at -70 °C within 5 days of collection. No supplements or preservatives were added. The fresh-frozen urine sample was allowed to thaw in transit to participants. The fresh-frozen urine sample is expected to be free of artifactual matrix effects, and therefore comparisons made between participants' results, or among method group mean/median values, will reflect performance expected for patients' samples. The ABU Survey always includes 3 different fresh-frozen pooled urine samples.

Urine Albumin, U-A 2018 Survey
U-06

Figure 1 shows the mean, lowest and highest values reported by 2173 participants for instrument/method groups with at least 10 results. Table 1 shows the instrument/method codes used in Figure 1.

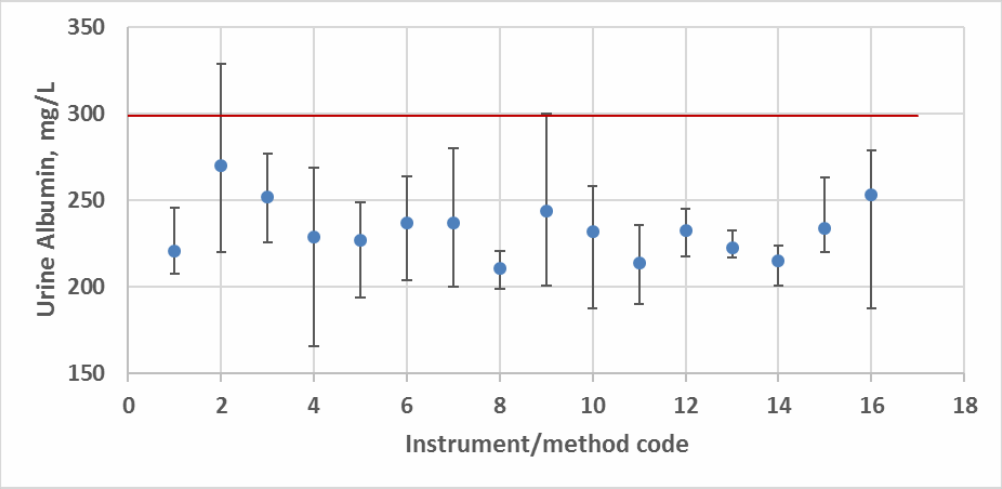

Figure 1. Urine albumin results for peer-groups with 10 or more results. The circle is the median and the limits represent the lowest and highest individual values reported. The solid line at 299

mg/L is the value obtained from an isotope dilution liquid chromatography mass spectrometry comparative method performed at the Mayo Clinic Renal Reference Laboratory.

CODE	INSTRUMENT	METHOD	NO LABS
1	BECKMAN IMMAGE	NEPHELOMETRY	19
2	SIEMENS DIMENSION VISTA	NEPHELOMETRY	339
3	SIEMENS NEPHELOMETR SYS	NEPHELOMETRY	20
4	SIEMENS DIMENSION	PETIA / PETINIA	117
5	SIEMENS DIMENSION Xpand	PETIA / PETINIA	10
6	ABBOTT ARCHITECT c	TURBIDIMETRC/IMMUNOTURB	266
7	BECKMAN AU SERIES	TURBIDIMETRC/IMMUNOTURB	254
8	BECKMAN AU/KAMIYA RGT	TURBIDIMETRC/IMMUNOTURB	18
9	BECKMAN UNICEL DxC SYST	TURBIDIMETRC/IMMUNOTURB	278
10	ROCHE COBAS c500 SER	TURBIDIMETRC/IMMUNOTURB	444
11	ROCHE COBAS c700 SER	TURBIDIMETRC/IMMUNOTURB	54
12	ROCHE COBAS INTEGRA	TURBIDIMETRC/IMMUNOTURB	28
13	ROCHE MODULAR	TURBIDIMETRC/IMMUNOTURB	15
14	SIEMENS ADVIA CHEM SYST	TURBIDIMETRC/IMMUNOTURB	61
15	SIEMENS DCA 2K/+,VANTAGE	TURBIDIMETRC/IMMUNOTURB	18
16	VITROS 5,1 FS/4600/5600	TURBIDIMETRC/IMMUNOTURB	232

Table 1. Instrument/method codes for Figure 1.

The urine albumin has an overall mean concentration of 233 mg/L with a 26% difference between the highest and lowest mean values and a 70% difference between the highest and lowest individual values reported. The Mayo Clinic Renal Reference Laboratory measured a value of 299 mg/L for the fresh-frozen sample with an isotope dilution liquid chromatography mass spectrometry (ID-LC/MS) measurement procedure being developed as a candidate reference measurement procedure. All of the mean values for the routine laboratory methods were biased low, -10% to -29%, compared to the ID-LC/MS value. These results are discordant with those from an earlier study that compared results for 332 non-frozen individual urine samples using many of the same routine methods compared to the same ID-LC/MS measurement procedure (1). This earlier study found that some routine methods had mean biases higher and some lower than the ID-LC/MS values with an approximate 40% difference in mean values among the method groups and an approximate 100% difference between the highest and lowest individual values reported.

The Laboratory Working Group of the National Kidney Disease Education Program has recommended method performance goals of bias ±13% vs a reference measurement procedure and a CV <6% (2). The 26% difference in mean values in the U-A Survey is consistent with acceptable agreement among the different routine methods. However, the low bias for all methods vs. the candidate reference measurement procedure along with the results in reference 1 indicate that substantial improvement is needed to meet the bias goal. The 70% difference between the highest and lowest individual values reported and the relatively large dispersion of individual results for some instrument/method groups suggests that uniformity of calibration in different laboratories also needs to be addressed by IVD manufacturers. Examination of the summary tables in the U-A participant summary shows the within instrument/method group CVs were 1.9-7.2%, indicating that most methods meet the precision goal but some need improvement.

Urine Albumin, ABU-A 2018 Survey

The Accuracy Based Urine Survey uses fresh-frozen urine for all samples, has candidate reference measurement procedure values, but has fewer participants (approximately 58). The median values for five peer groups were -26% to -2% at 16 mg/L; -12% to 4% at 36 mg/L; and -14% to -1% at 184 mg/L vs the ID-LC/MS measurement procedure values. The lowest to highest urine albumin results were 111% different at 16 mg/L, 81% different at 36 mg/L and 171% different at 184 mg/L. The biases observed in the ABU-A 2018 Survey are generally similar to those from earlier ABU Surveys. The differences between the lowest and highest individual values in the ABU-A Survey are much larger than the 70% difference observed in the U-A Survey at 233 mg/L. Although the ABU Survey had far fewer participants, the differences between the largest and smallest individual values reported should be representative of current performance in clinical laboratories. Larger biases at lower urine albumin concentrations in the ABU-A Survey are consistent with a large study that used 332 individual urine samples and observed larger biases at lower urine albumin concentrations (3).

The differences in results observed among urine albumin methods in each of these Surveys are large enough to affect risk classification and treatment decisions for people with kidney disease. Clearly there is a need for improved standardization of urine albumin results. The Laboratory Working Group of the National Kidney Disease Education Program is developing higher order certified reference materials and reference measurement procedures to improve the agreement of results among different clinical laboratory measurement procedures (3). Surveys using fresh-frozen urine samples are important for surveillance of performance in clinical laboratories. Laboratories are encouraged to participate in the ABU Survey as a supplement to the U Survey to improve the information available to monitor performance.

Urine Total Protein, U-A 2018 Survey
U-03

Figure 2 shows the mean, lowest and highest values reported by 2917 participants for instrument/method groups with at least 10 results. Table 2 shows the instrument/method codes used in Figure 2.

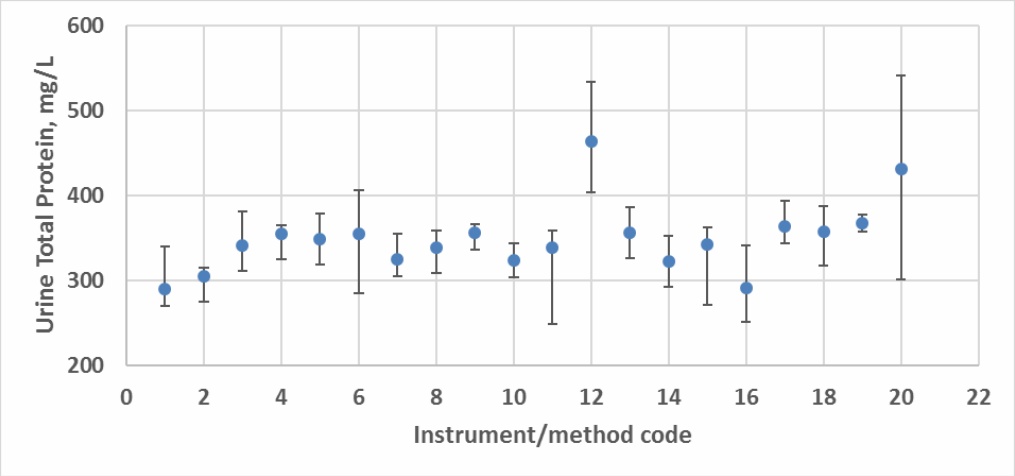

Figure 2. Urine total protein results for peer-groups with 10 or more results. The circle is the mean and the limits represent the lowest and highest individual values reported.

CODE	INSTRUMENT	METHOD	NO LABS
1	ROCHE COBAS c500 SER	BENZETH Cl (ROCHE OUS)	89
2	ROCHE COBAS c700 SER	BENZETH Cl (ROCHE OUS)	14
3	ABBOTT ARCHITECT c	BENZETHONIUM CHLORIDE	310
4	ROCHE COBAS c311	BENZETHONIUM CHLORIDE	11
5	ROCHE COBAS c500 SER	BENZETHONIUM CHLORIDE	434
6	ROCHE COBAS c700 SER	BENZETHONIUM CHLORIDE	44
7	ROCHE MODULAR	BENZETHONIUM CHLORIDE	11
8	ABBOTT ARCHITECT c	BIURET	63
9	BECKMAN AU SERIES	BIURET	19
10	BECKMAN UNICEL DxC SYST	BIURET	28
11	ROCHE COBAS c500 SER	BIURET	59
12	VITROS 5,1 FS/4600/5600	PYROCATECHOL VIOLET	284
13	BECKMAN AU SERIES	PYROGALLOL RED	355
14	BECKMAN UNICEL DxC SYST	PYROGALLOL RED	318
15	ROCHE COBAS c500 SER	PYROGALLOL RED	20
16	SIEMENS ADVIA CHEM SYST	PYROGALLOL RED	72
17	SIEMENS DIMENSION	PYROGALLOL RED	234
18	SIEMENS DIMENSION VISTA	PYROGALLOL RED	505
19	SIEMENS DIMENSION Xpand	PYROGALLOL RED	10
20	VITROS 5,1 FS/4600/5600	PYROGALLOL RED	37

Table 2. Instrument/method codes for Figure 2.

The data for urine total protein at an approximate concentration of 350 mg/L has a 50% difference between the highest and lowest mean values and an 84% difference between the highest and lowest individual values reported. Excluding results for the Vitros methods (codes 12 and 20) the mean values differ by 23% and the difference between the highest and lowest individual values is 47%. Examination of the summary tables in this participant summary shows the within instrument/method group CVs are 1.7-13.2%. The measurement limitation with urine total protein methods is that different chemical reactions give different measurement responses to different proteins and each method has a different response ratio to different mixtures of proteins in a urine sample. Four types

of chemical reactions were represented in the U-A Survey and, with exception of the Vitros implementations, the mean results had reasonably good agreement among the different instrument/method groups. However, results at the relatively low urine protein value in the fresh-frozen sample are not likely representative of the much larger concentrations and different types of proteins seen in urine from patients with advanced kidney disease.

Urine Total Protein, ABU-A 2018 Survey

The ABU-A Survey had 31 participants who reported results for urine total protein. Only two method groups were represented in the lower concentration samples and three for the 260 mg/L sample. The median values were reasonably close to each other but from too few participants for meaningful comparison. The lowest to highest individual results were 114% and 185% different for two samples with approximately 70 mg/L total protein, and 141% different at 260 mg/L. The ABU-A results for urine total protein are consistent with those from the U-A Survey and show that urine total protein measurements vary substantially among different methods.

There is no current activity to standardize urine total protein methods because there is not a suitable protein molecule to use as a reference material for calibration and the mix of different proteins in urine make it impossible to optimize the current methodologies for a uniform response to concentrations of different proteins. Despite the limitations in current measurement methods, urine total protein is an important parameter for monitoring kidney disease and other diseases that produce various amounts and different types of proteins in urine. However, urine albumin is preferred and recommended for identification of early chronic kidney disease, assessing risk for cardiovascular disease and monitoring for progression of chronic kidney disease (4).

Urine Creatinine, U-A 2018 Survey U-06

Figure 3 shows the mean and ±2 SD reported by 3311 participants for instrument/method groups with at least 10 results. The method groups are in the sequence shown in the participant summary.

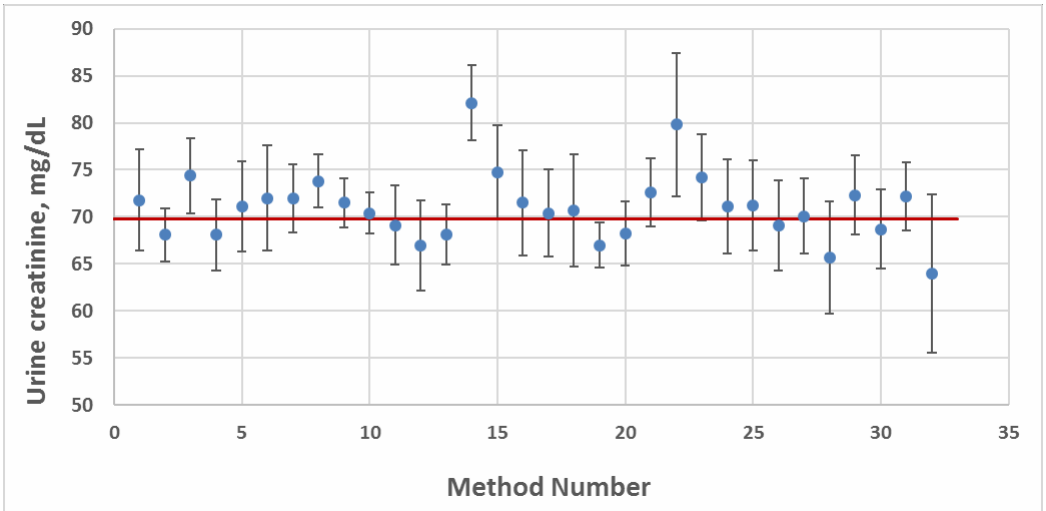

Figure 3. Urine creatinine results for peer-groups with 10 or more results. The circle is the mean and the limits represent ±2 SD for the distribution of individual results in each method group. The solid line at 70 mg/dL is the value obtained from an isotope dilution liquid chromatography mass spectrometry reference measurement procedure at the Centers for Disease Control and Prevention (CDC).

If the results for the two largest, Beckman AU Jaffe methods, and one lowest, Dimension Xpand Jaffe method, mean values are ignored, the remaining 2897 results have a 13% difference between the largest and smallest mean values, and a 28% difference between the 2SD limits. These collective results for 29 method groups meet the desirable total allowable error specifications, 27.8%, for an individual method based on the biological variability model for a first morning urine sample (5). Standardization of results for urine creatinine measurement appears to be adequate, at least at concentrations near 70 mg/dL. Two IVD manufacturers with either high or low biased results should review their calibration procedures to become aligned with the reference measurement procedure.

Urine Creatinine, ABU-A 2018 Survey

The ABU-A Survey had 67 participants who reported results for urine creatinine. The median values for all six peer groups were -10% to 11% at 55 mg/dL; -5% to 14% at 67 mg/dL; and -9% to 10% at 88 mg/dL vs the ID-LC/MS reference measurement procedure values from the CDC. Removing results for the Beckman AU method group that showed high bias in the ABU-A Survey, the median values for five remaining peer groups were -10% to -2.5% at 55 mg/dL; -5% to 1.5% at 67 mg/dL; and -9% to 0% at 88 mg/dL vs the ID-LC/MS reference measurement procedure. Without the method group with obvious high bias, the lowest to highest individual results were 7.5% different at 55 mg/dL, 7.0% different at 67 mg/dL and 9.5% different at 88 mg/dL. The ABU-A results for urine creatinine are consistent with those from the U-A Survey and confirm that standardization of results

for urine creatinine measurement appears to be adequate at commonly encountered concentrations in urine for almost all instrument/methods represented in these two Surveys.

References

1. Bachmann LM, Nilsson G, Bruns DE, et al. State of the art for measurement of urine albumin: comparison of routine measurement procedures to isotope dilution tandem mass spectrometry. *Clin Chem*. 2014;60:471-80.

2. Miller WG, Seegmiller JC, Lieske JC, et al. Standardization of Urine Albumin Measurements: Status and Performance Goals. *J Applied Lab Med*. 2017;2:423-9.

3. Seegmiller JC, Miller WG, Bachmann LM. Moving towards standardization of urine albumin measurements. *eJIFCC*. 2017;28:258-67.

4. KDIGO Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Kidney Int Suppl. 2013;3:1-150.

5. Desirable Biological Variation Database specifications. https://www.westgard.com/biodatabase1.htm. Accessed 8.13.2018.

Greg Miller, PhD
Accuracy Based Testing Committee