Moving toward standardization of urine albumin measurements

Jesse C. Seegmiller[1], W. Greg Miller[2], Lorin M. Bachmann[2]

[1] University of Minnesota, Department of Laboratory Medicine and Pathology, Minneapolis, Minnesota, USA
[2] Virginia Commonwealth University, Department of Pathology, Richmond, Virginia, USA

ARTICLE INFO

Corresponding author:
Jesse C. Seegmiller
University of Minnesota
Department of Laboratory Medicine
and Pathology
420 Delaware Street S.E.
Minneapolis, MN 55455
United States of America
Phone: 1-612-625-6198
Fax: 1-612-625-4831
E-mail: jseegmil@umn.edu

ABSTRACT

Measurement of urine albumin is important for detecting and monitoring kidney disease. At the present time, measurement of urine albumin is not standardized due to the lack of a reference system, which includes both a reference measurement procedure and certified reference materials. Developing a reference system will provide a means for clinical laboratory measurement procedures to become standardized and will enable successful use of uniform clinical decision points. Currently, urine albumin results vary in excess of 40% depending on which commercially available measurement procedure is utilized for measurement. Clinicians may struggle with classification of kidney disease in part due to differences in measurements from lack of agreement among laboratory methodologies employed when assessing urine albumin concentrations. This report focuses on current findings in urine albumin testing, highlights important measurement and reporting considerations, and presents strategies for developing a reference measurement procedure to enable standardization of urine albumin measurements.

INTRODUCTION

Urine albumin is a diagnostic and prognostic marker for chronic kidney disease (CKD), diabetes and cardiovascular disease.[1,2] When interpreting measurements of urine albumin, providers must consider the type of urine collection and the methodology used for analytical measurement. The historical standard for measuring the amount of albumin excreted into the urine, known as the urine albumin excretion rate, has been to measure the albumin concentration obtained from a 24-hour urine collection.[3] In clinical practice, 24-hour urine collections present problems in terms of specimen storage and timing accuracy. Thus, assessment of urine albumin from shorter collection times is a common clinical practice and presents a more convenient collection option. In untimed situations, the urine albumin result should be indexed to urine creatinine concentration and reported as the albumin to creatinine ratio (ACR). The ACR accounts for hydration and produces a ratio that has similar diagnostic performance as a 24-hour urine albumin excretion rate.[4-6] A caveat to these different timing approaches is differences in classification of albuminuria depending on timing of collection. Therefore, the collection method should remain consistent throughout studies.[7] Recommendations are to report the ACR along with the albumin concentration, preferably collect the first morning void specimen, and follow-up findings from random urine collections with first morning void collections.[4,8-10]

A variety of testing methodologies have been employed to monitor urine albumin including turbidimetry[11,12], dipstick[13], radioimmunoassay[14,15], immunoturbidimetry[16], immunonephelometry[17,18], high performance liquid chromatography[19], liquid chromatography mass spectrometry[20,21], and liquid chromatography tandem mass spectrometry (LC-MS/MS)[22].

Some of these methods are known to have issues with analytical specificity when measuring urine albumin. One essential attribute for a reference measurement procedure is that it must be specific for the measurand it is intended to quantify and not be influenced by matrix effects or interfering substances that can be present in patient urine.

This report highlights standardization recommendations for urine albumin measurements and focuses on methodologies likely suitable for use as a reference measurement procedure for standardizing such measurement results.

PREANALYTICAL AND STORAGE CONSIDERATIONS FOR URINE ALBUMIN

Several precollection factors have been shown to increase urine albumin excretion such as exercise[23], posture[24] and fever[25]. These factors should be considered when assessing albuminuria for comprehensive renal workups. Interventions may not be indicated in patients with the above conditions unless the albuminuria persists when the confounding physiological conditions are no longer present. Nonspecific binding of albumin to urine collection containers does not contribute to measurement error, as binding to the container has been estimated to be <1% depending on the container hydrophobicity, which is considered inconsequential.[26]

A fresh midstream collection for urine albumin measurement is preferred.[8,27,28] Albumin can remain stable in urine for up to 8 weeks when stored under refrigerated conditions at 4 °C.[29] For long term frozen storage of urine albumin samples, a temperature -70 °C or lower is required. Degradation of albumin in urine causing measurement issues has been reported when stored at -20 °C over periods of 2 weeks to 3 years.[29,30] Therefore, careful attention must be paid to the storage conditions for urine specimens particularly for investigations using stored samples.

CURRENT STATE OF URINE ALBUMIN MEASUREMENTS

While the utility of this biomarker is clear, applying disease specific cutoffs for albuminuria becomes compromised near the decision values due to non-standardized measurement procedures used in clinical laboratories. In a study that evaluated the state of agreement among 16 quantitative clinical laboratory immunoassay measurement procedures from in-vitro diagnostics manufacturers, who distribute globally, results from 332 freshly collected non-frozen urine albumin samples had total coefficients of variation (CVs) of 5.2-8.1% and the effects of sample-specific influences were < 10% for most measurement procedures.[31]

However, bias was found to cause a significant lack of agreement among measurement procedures. The median difference range for routine measurement procedures vs. a comparator LC-MS/MS procedure was approximately 40%. Mean biases ranged from -35% to +34% for concentrations near 15 mg/L and -15% to +18% for concentrations near 30 mg/L. The results of this study demonstrate that fixed decision thresholds cannot be effectively utilized due to lack of agreement among routine measurement procedures and therefore standardization is needed.

The College of American Pathologists offers an Accuracy Based Urine Survey that uses unaltered pooled frozen human urine as the samples.

Table 1	Results from the College of American Pathologists - Accuracy Based Urine Survey first mailing in 2017[a]					
Sample	Methods	N. Labs	Median, mg/L	Median bias vs. LC-MS/MS, %	Low value, mg/L	High value, mg/L
A	Siemens Dimension Vista (IN)	7	16	-1.8	16	18
	Abbott Architect c Systems (IT)	10	13	-20.2	11	13
	Beckman AU Series (IT)	8	13	-20.2	11	14
	Roche cobas c500 Series	9	12	-26.4	10	13
	Vitros 5.1 FS/4600/5600	5	15	-8.0	8	16
	All methods	58	13	-20.2	8	18
	LC-MS/MS	-	16.3	-	-	-

B	Siemens Dimension Vista (IN)	7	38	4.1	37	38
	Abbott Architect c Systems (IT)	10	32	-12.3	30	33
	Beckman AU Series (IT)	7	31	-15.1	30	32
	Roche cobas c500 Series	11	32	-12.3	30	34
	Vitros 5.1 FS/4600/5600	5	37	1.4	25	38
	All methods	59	32	-12.3	25	39
	LC-MS/MS	-	36.5	-	-	-

C	Siemens Dimension Vista (IN)	7	192	4.1	178	195
	Abbott Architect c Systems (IT)	9	164	-11.1	161	167
	Beckman AU Series (IT)	7	167	-9.4	149	169
	Roche cobas c500 Series	11	155	-15.9	130	173
	Vitros 5.1 FS/4600/5600	5	166	-10.0	133	180
	All methods	58	164	-11.1	130	195
	LC-MS/MS	-	184.4	-	-	-

[a] Data used with permission from the College of American Pathologists
(IN) - immunonephelometric, (IT) - immunoturbidimetric

Table 1 shows participant results compared to an LC-MS/MS candidate reference measurement procedure. Although there were a small number of participants, the information is representative and consistent with the previously mentioned larger study based on individual patient urine samples.[31] The median bias vs. the comparative method was larger at lower concentrations of urine albumin with the all methods bias -20% at 16 mg/L, -12% at 36 mg/L, and -11% at 184 mg/L. The joint committee of the Laboratory Working Group of the National Kidney Disease Education Program and the International Federation of Clinical Chemistry and Laboratory Medicine Working Group for Standardization of Albumin in Urine recommended desirable and optimal bias goals of ≤13% and ≤7%, respectively, vs. a reference measurement procedure.[32]

These survey results suggest that some measurement procedures can meet these bias goals, but many do not. The survey also reported ACR values. Reference measurement procedure results were not available for urine creatinine but comparison of mean results among different methods in the survey had differences of 17%, 8.8% and 14% at mean concentrations of 55 mg/dL, 69 mg/dL and 89 mg/dL (4.8 mmol/L, 6.1 mmol/L and 7.9 mmol/L), respectively. When both creatinine and albumin were used to calculate the ACR, the differences between the lowest and highest ACR values for all methods combined were 76% at 15 mg/g, 49% at 60 mg/g, and 65% at 237 mg/g. These differences will cause misclassification of risk of kidney disease at the commonly used albuminuria decision values of 30 and 300 mg/g creatinine (3.4 and 34 mg/mmol creatinine).

A reference system is in place for urine creatinine and perhaps needs to be more stringently implemented. However, a reference system does not yet exist for urine albumin and is the focus of this report.

METHODS FOR MEASURING URINE ALBUMIN

To improve the analytical selectivity in the measurement of urine albumin, liquid chromatography mass spectrometry (LC-MS) methods were utilized.[20,21] A comparison study of one LC-MS method to an immunoturbidimetric method found the comparability between the methodologies greatly improved when both methods employed the same calibrators with the same calibrator value assignments. Mean bias improved from -37.8% to 2.2% using the same calibrators on both platforms.[33] A potential shortcoming of the LC-MS urine albumin method was the lower limit of quantitation of 10-20 mg/L, which is above the level expected in specimens with normal albumin concentrations.[20,33] Other possibilities that could introduce error with this methodology are the presence of urine albumin fragments containing the N-terminal fragment used in the analysis, which could falsely elevate albumin levels or modification to the N-terminal portion used in analysis that would change the mass, which could falsely lower albumin levels.

In an effort to improve the lower limit of quantitation for urine albumin, a LC-MS/MS method was developed.[22] This method employed proteolysis of urinary proteins to produce peptides of albumin as well as peptides from other proteins present in urine. Large variations in pH (4.5-8) and specific gravity are expected in the urine of patients with or without a kidney abnormality.[34] pH variations could adversely affect the trypsin proteolysis process, which is a critical preanalytic step that occurs prior to LC-MS/MS measurement. Therefore, buffering conditions and dilutions were employed that provide an optimal environment for trypsin proteolysis. Peptides known to be unique to albumin were analyzed and quantitated to represent the quantity of intact albumin. One

of the key components of this method was the incorporation of an internal standard that consisted of a recombinant form of human serum albumin isotopically labeled with [15]N.

The internal standard served dual purpose:

1. to normalize for any differences in the proteolytic processing among specimens;

2. to provide normalization for LC-MS/MS analysis.

Several peptides unique to human serum albumin were quantitated and referenced to a calibration curve. The lower limit of quantitation for the LC-MS/MS measurement procedure was found to be 3.13 mg/L.[22] Method comparison studies have been performed examining commercially available immunoassay platforms to the LC-MS/MS method.[31,35] The LC-MS/MS measurement procedure was used to perform the comparison study of 16 commercially available measurement procedures previously described.

Potential challenges for a LC-MS/MS method include the possibility of albumin fragments in the urine, post-translational modifications of the unique peptides monitored, or factors that inhibit albumin proteolysis. Further investigation of this technique compared urine albumin concentrations before and after ultrafiltration using a 10 kDa molecular weight cutoff filter where differences in the results were small and suggested minimal signal contribution from fragments of albumin.[36] With the above cautions appropriately addressed in the measurement procedure details, the LC-MS/MS method is a good candidate reference measurement procedure for urine albumin. This method provides the necessary sensitivity to assess urine albumin concentrations <5 mg/L. The ability to quantitate the albumin molecule with a high degree of analytical specificity by using proteotypic peptides of albumin that are not known to be subject to modification and

do not appear in other human proteins, provides support for use of the LC-MS/MS method as a reference measurement procedure. To ensure high quality results, the LC-MS/MS measurement procedure requires an isotopically enriched form of albumin as an internal standard. Procedures for making labeled albumin have been described.[21]

A HIGHER ORDER REFERENCE SYSTEM FOR CALIBRATION TRACEABILITY

A higher order reference system is needed to enable all measurement procedures to implement common calibration traceability to achieve equivalent results for urine albumin irrespective of the measurement procedure used. A reference system for urine albumin that follows the International Organization for Standardization standard 17511 for calibration traceability hierarchy[37] includes three main components:

1. A pure human albumin primary reference material.

2. A reference measurement procedure.

3. A human urine matrix based secondary reference material.

The National Institute for Standards and Technology (NIST) in the USA is qualifying a recombinant human albumin certified primary reference material expected to be released in 2018 as SRM 2925. SRM 2925 will be a highly purified solid substance intended to be used to prepare calibrators for a mass spectrometry based reference measurement procedure. SRM 2925 is not intended to be used to prepare calibrators for immunoassays. NIST is also preparing an albumin in frozen human urine certified reference material designated SRM 3666 that will include four concentrations intended to be used to establish the metrological traceability of calibration for clinical laboratory measurement procedures, including immunoassays.

SRM 3666 will be value assigned using a NIST reference measurement procedure that is currently in development. The commutability of NIST SRM 3666 will be validated to ensure it is suitable for use as a calibrator for manufacturer's selected measurement procedures as well as for clinical laboratory measurement procedures. It is not known at this time when either the reference measurement procedure or the SRM 3666 will be available from NIST.

Since SRM 2925 pure albumin will be available soon, development of suitable reference measurement procedures will provide the essential components of a reference system to allow standardized calibration traceability for commercially available clinical laboratory urine albumin immunoassay procedures.

A reference measurement procedure intended for use in a calibration traceability hierarchy for clinical laboratory measurement procedures must have performance characteristics to ensure acceptable uncertainty in values assigned to patient samples used as calibrators in the traceability hierarchy, as described below. In addition, a reference measurement procedure must be operational in at least two sites to

Figure 1	Metrologic traceability hierarchy for calibration of urine albumin measurement procedures
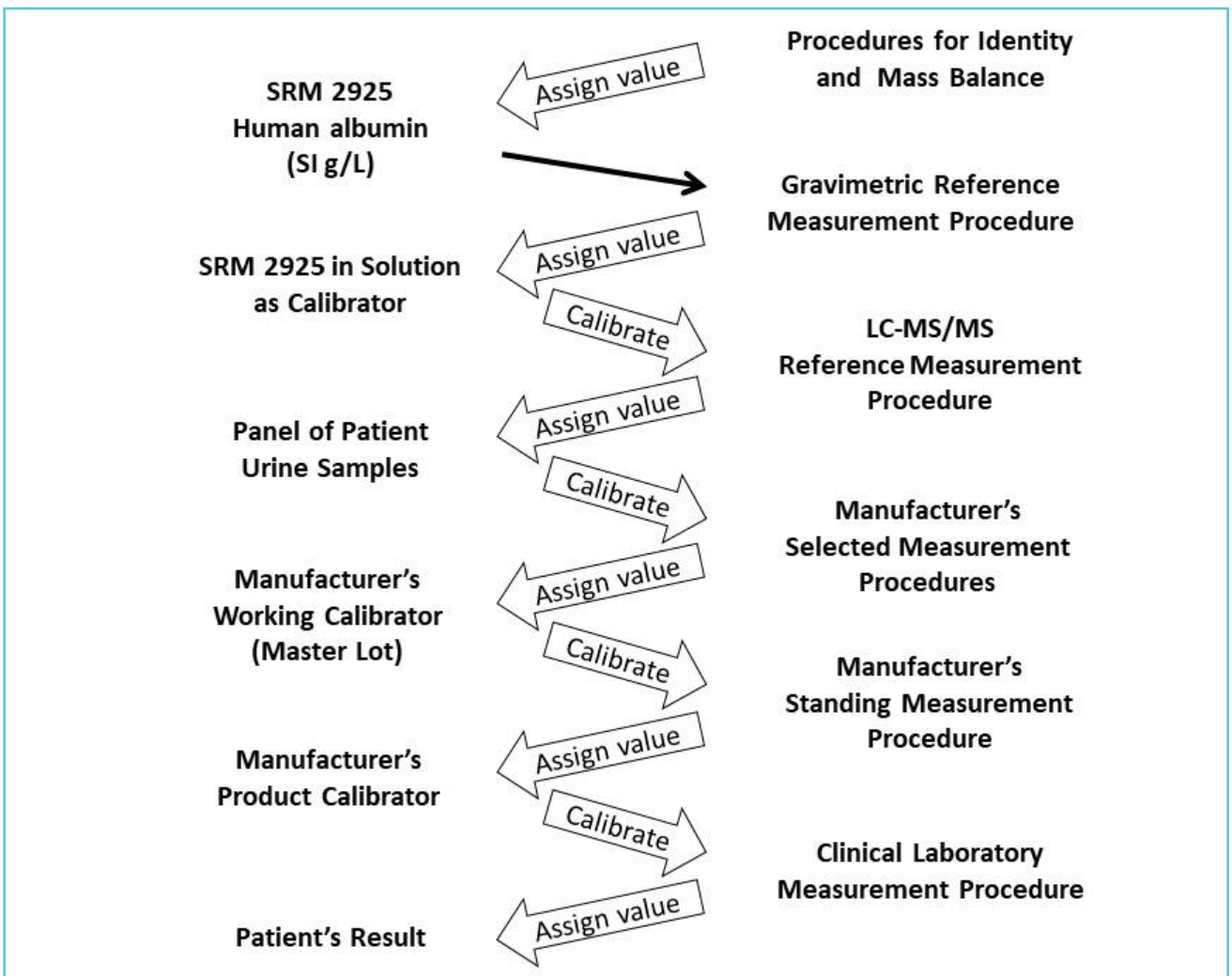
validate equivalent performance to qualify for listing by the Joint Committee for Traceability in Laboratory Medicine.

Metrologic traceability of calibration is described in the International Organization for Standardization 17511 standard.[37] Figure 1 shows how the reference system components being developed for urine albumin fit into the traceability hierarchy. NIST SRM 2925 is a pure substance primary reference material that is used with a gravimetric reference measurement procedure to prepare calibrators for an LC-MS/MS reference measurement procedure. The LC-MS/MS reference measurement procedure is used to assign values to a panel of patient's urine samples that are used as calibrators for a manufacturer's selected measurement procedure that is used to assign values to the manufacturer's working, or master lot, calibrator.

In the case of urine albumin, there will be several concentrations of working calibrator used to calibrate the manufacturer's standing immunoassay measurement procedure. The working calibrators can be prepared as dilutions of a single master lot of working calibrator or as a set of concentrations of working calibrators, with each value assigned by the selected measurement procedure. The manufacturer's standing measurement procedure is then used to value assign sequential lots or batches of the manufacturer's product calibrator that is used to calibrate the clinical laboratory measurement procedures. In many cases, the manufacturer's selected and standing measurement procedures will be the same as the clinical laboratory measurement procedure but operated with a more stringent protocol for items such as maintenance, calibration, replicate measurements, multiple reagent lots and/or instruments to reduce the uncertainty of the value assignment steps. Thus, metrologic traceability is established from patient results to the pure substance primary reference material.

When NIST SRM 3666, albumin in frozen human urine, becomes available it can replace the panel of patient urine samples to simplify the traceability process. In addition, SRM 3666 can be used by clinical laboratories to verify calibration of their immunoassay measurement procedures for urine albumin.

CONCLUSIONS

The need for standardization of urine albumin measurements is clear. Standardization of this measurand will assist in applying uniform clinical decision points for various diseases and conditions based on urine albumin to creatinine ratio values. Standardization of urine albumin measurements requires development of both a certified primary reference material and a reference measurement procedure. LC-MS/MS measurement of albumin-specific peptides after proteolytic digestion under carefully controlled conditions provides a suitable methodology for a reference measurement procedure. When available, these reference system components can be used by immunoassay measurement procedure manufacturers to achieve metrologic traceability of calibration to a common reference system. Availability of a commutable frozen human urine reference material will also be useful as a common calibrator for immunoassays. In addition to standardized metrologic traceability, urine collection and storage conditions influence the suitability of urine albumin measurements and therefore preanalytical processing procedures should be standardized.

REFERENCES

1. Mogensen CE. Microalbuminuria predicts clinical proteinuria and early mortality in maturity-onset diabetes. N Engl J Med. 1984;310(6):356-360.

2. Brantsma AH, Bakker SJ, Hillege HL, de Zeeuw D, de Jong PE, Gansevoort RT. Cardiovascular and renal outcome in subjects with K/DOQI stage 1-3 chronic kidney disease: the importance of urinary albumin excretion. Nephrol Dial Transplant. 2008;23(12):3851-3858.

3. Keane WF, Eknoyan G. Proteinuria, albuminuria, risk, assessment, detection, elimination (PARADE): a position paper of the National Kidney Foundation. Am J Kidney Dis. 1999;33(5):1004-1010.

4. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Kidney International. 2013;Suppl(3):1-150.

5. Witte EC, Lambers Heerspink HJ, de Zeeuw D, Bakker SJ, de Jong PE, Gansevoort R. First Morning Voids Are More Reliable Than Spot Urine Samples to Assess Micro-albuminuria. In: J Am Soc Nephrol. Vol 20.2009:436-443.

6. Lambers Heerspink HJ, Gansevoort RT, Brenner BM, et al. Comparison of different measures of urinary protein excretion for prediction of renal events. J Am Soc Nephrol. 2010;21(8):1355-1360.

7. Younes N, Cleary PA, Steffes MW, et al. Comparison of urinary albumin-creatinine ratio and albumin excretion rate in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications study. Clin J Am Soc Nephrol. 2010;5(7):1235-1242.

8. Miller WG, Bruns DE, Hortin GL, et al. Current issues in measurement and reporting of urinary albumin excretion. Clin Chem. 2009;55(1):24-38.

9. Saydah SH, Pavkov ME, Zhang C, et al. Albuminuria prevalence in first morning void compared with previous random urine from adults in the National Health and Nutrition Examination Survey, 2009-2010. Clin Chem. 2013;59(4):675-683.

10. Hortin GL. Identifying optimal sample types and decision thresholds for the urinary albumin-creatinine ratio. In: Clin Chem. Vol 59. United States2013:598-600.

11. Folin O, Denis W. The Quantitative Determination of Albumin in Urine. J Biol Chem. 1914;18:273-276.

12. Exton WG. A Simple and Rapid Test for Albumin and other Urinary Proteins. JAMA. 1923;80(8):529-530.

13. Free AH, Rupe CO, Metzler I. Studies with a new colorimetric test for proteinuria. Clin Chem. 1957;3(6):716-727.

14. Keen H, Chlouverakis C. An Immunoassay Method for Urinary Albumin at Low Concentrations. Lancet. 1963;2(7314):913-914.

15. Woo J, Floyd M, Cannon DC, Kahan B. Radioimmunoassay for urinary albumin. Clin Chem. 1978;24(9):1464-1467.

16. Teppo AM. Immunoturbidimetry of albumin and immunoglobulin G in urine. Clin Chem. 1982;28(6):1359-1361.

17. Ellis D, Buffone GJ. New approach to evaluation of proteinuric states. Clin Chem. 1977;23(4):666-670.

18. Marre M, Claudel JP, Ciret P, Luis N, Suarez L, Passa P. Laser immunonephelometry for routine quantification of urinary albumin excretion. Clin Chem. 1987;33(2 Pt 1):209-213.

19. Comper WD, Osicka TM, Jerums G. High prevalence of immuno-unreactive intact albumin in urine of diabetic patients. Am J Kidney Dis. 2003;41(2):336-342.

20. Babic N, Larson TS, Grebe SK, Turner ST, Kumar R, Singh RJ. Application of liquid chromatography-mass spectrometry technology for early detection of microalbuminuria in patients with kidney disease. Clin Chem. 2006;52(11):2155-2157.

21. Singh R, Crow FW, Babic N, et al. A liquid chromatography-mass spectrometry method for the quantification of urinary albumin using a novel 15N-isotopically labeled albumin internal standard. Clin Chem. 2007;53(3):540-542.

22. Seegmiller JC, Barnidge DR, Burns BE, Larson TS, Lieske JC, Kumar R. Quantification of urinary albumin by using protein cleavage and LC-MS/MS. Clin Chem. 2009;55(6):1100-1107.

23. Poortmans J, Dorchy H, Toussaint D. Urinary Excretion of Total Proteins, Albumin, and β2-Microglobulin During Rest and Exercise in Diabetic Adolescents With and Without Retinopathy. Diabetes Care. 1982.

24. Uehara K, Tominaga N, Shibagaki Y. Adult orthostatic proteinuria. Clin Kidney J. 2014;7(3):327-328.

25. Hemmingsen L, Skaarup P. Urinary excretion of ten plasma proteins in patients with febrile diseases. Acta Med Scand. 1977;201(4):359-364.

26. Robinson MK, Caudill SP, Koch DD, et al. Albumin adsorption onto surfaces of urine collection and analysis containers. Clin Chim Acta. 2014;431:40-45.

27. CLSI GP16-A3 Urinalysis; Approved Guideline-Third Addition. In: Institute CaLS, ed. Third ed. Wayne, PA2000.

28. European urinalysis guidelines. Scand J Clin Lab Invest Suppl. 2000;231:1-86.

29. Osberg I, Chase HP, Garg SK, et al. Effects of storage time and temperature on measurement of small concentrations of albumin in urine. Clin Chem. 1990;36(8 Pt 1):1428-1430.

30. Sviridov D, Drake SK, Hortin GL. Reactivity of urinary albumin (microalbumin) assays with fragmented or modified albumin. Clin Chem. 2008;54(1):61-68.

31. 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(3):471-480.

32. Miller WG, Seegmiller JC, Lieske JC, Narva AS, Bachmann LM. Standardization of Urine Albumin Measurements:

Status and Performance Goals. The Journal of Applied Laboratory Medicine. 2017;2(3):423-429.

33. Shaikh A, Seegmiller JC, Borland TM, et al. Comparison between Immunoturbidimetry, Size-Exclusion Chromatography, and LC-MS to Quantify Urinary Albumin. Clin Chem. 2008;54(9):1504-1510.

34. Simerville JA, Maxted WC, Pahira JJ. Urinalysis: a comprehensive review. Am Fam Physician. 2005; 71(6):1153-1162.

35. Seegmiller JC, Sviridov D, Larson TS, Borland TM, Hortin GL, Lieske JC. Comparison of urinary albumin quantification by immunoturbidimetry, competitive immunoassay, and protein-cleavage liquid chromatography-tandem mass spectrometry. Clin Chem. 2009;55(11):1991-1994.

36. Lieske JC, Bondar O, Miller WG, et al. A reference system for urinary albumin: current status. Clin Chem Lab Med. 2013;51(5):981-989.

37. ISO 17511:2003 - In vitro diagnostic medical devices -- Measurement of quantities in biological samples -- Metrological traceability of values assigned to calibrators and control materials. International Organization for Standardization, Geneva, Switzerland 2003.