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Reviews

Current Issues in Measurement and Reporting of Urinary Albumin Excretion

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BACKGROUND: Urinary excretion of albumin indicates kidney damage and is recognized as a risk factor for progression of kidney disease and cardiovascular disease. The role of urinary albumin measurements has focused attention on the clinical need for accurate and clearly reported results. The National Kidney Disease Education Program and the IFCC convened a conference to assess the current state of preanalytical, analytical, and postanalytical issues affecting urine albumin measurements and to identify areas needing improvement.

CONTENT: The chemistry of albumin in urine is incompletely understood. Current guidelines recommend the use of the albumin/creatinine ratio (ACR) as a surrogate for the error-prone collection of timed urine samples. Although ACR results are affected by patient preparation and time of day of sample collection, neither is standardized. Considerable intermethod differences have been reported for both albumin and creatinine measurement, but trueness is unknown because there are no reference measurement procedures for albumin and no reference materials for either analyte in urine. The recommended reference intervals for the ACR do not take into account the large intergroup differences in creatinine excretion (e.g., related to differences in age, sex, and ethnicity) nor the continuous increase in risk related to albumin excretion.

DISCUSSION: Clinical needs have been identified for standardization of (*a*) urine collection methods, (*b*) urine albumin and creatinine measurements based on a complete reference system, (*c*) reporting of test results, and (*d*) reference intervals for the ACR.

Background

Urine albumin measurements are widely used to identify and monitor patients with kidney damage. A conference on the clinical use and measurement of urine albumin was organized by the Laboratory Working Group of the National Kidney Disease Education Program and the IFCC to examine the current practices in measuring urine albumin and using the resulting data in kidney disease management. The conference objectives were to increase understanding of the issues that must be addressed to enable standardization of measurements and clinical practice guidelines based on urine albumin excretion. This report summarizes the observations and conclusions from that conference.

Historically, albuminuria has been defined in terms of urinary excretion of albumin per unit time, typically 24 h. The difficulty of collecting 24-h urine samples has led to surrogate measurements of albumin excretion rate (AER).[13] A commonly used surrogate is the ratio of urinary concentrations of albumin and creatinine (ACR) *(1)*. Both of these surrogate measurements are considered in this report. For the ACR, random or spot urine samples often have been collected

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[13] Nonstandard abbreviations: AER, albumin excretion rate; ACR, albumin/creatinine ratio; CVi, within-subject biological variability; EQAS, external quality assessment schemes; RM, reference material; RMP, reference measurement procedure; JCTLM, Joint Committee for Traceability in Laboratory Medicine.

without regard to time of day, and a common reference interval is often cited for both men and women. Time of day affects results, however, and the higher excretion of creatinine in men than in women also affects the use of ACR as a surrogate for AER *(1)*, as do such things as the greater excretion of creatinine in blacks than in whites, the decreased excretion with muscle wasting, and the effects of diet on creatinine excretion.

Survey of Guidelines on Urine Albumin Use

Clinical practice guidelines for the use of urine albumin measurements have been issued by professional organizations in several countries. These guidelines are not uniform in recommendations regarding sample type, time of sample collection, units of reporting, reference intervals or cut points used for interpretation, nor methods used to measure albumin and creatinine.

Table 1 lists 10 guidelines, recommendations, and position statements issued by 7 organizations since 2002; 5 guidelines are diabetes related and recommend annual testing. All 10 recommend use of the ACR, 8 recommend early or first morning urine collections, 7 recommend random or spot urine collections, and 4 identify milligrams per gram or milligrams per millimole as the units of measure for ACR (1 mg/g = 1 μg/mg = 0.113 mg/mmol). Seven mention, with varying levels of detail, the need to carry out follow-up testing to confirm findings. Two explicitly state that 24-h urine collection is not needed, but 1 guideline lists 24-h collection as the first of 3 possible methods for collecting urine.

Although ACR measurement is commonly recommended, the absence of recognized standard methods for collecting samples, measuring ACR, and reporting results compromises the utility of this test in clinical and research settings. Reported results may be milligrams albumin per gram (or μg/mg) or per millimole of creatinine, and the meaning of neither is intuitively obvious to nonspecialists. Clinically, healthcare providers may not understand the meaning of albuminuria and may have difficulty interpreting results effectively (e.g., identifying results that indicate increased risk for cardiovascular disease or progression of kidney disease). Providers may be confused by too many options for kidney testing and be concerned that a single cutoff value, such as the commonly cited 30 mg/g (30 μg/mg, 3.4 mg/mmol, or 3.4 g/mol), may not be useful for patients of all ages, sexes, and ethnicities *(1, 4)*. Furthermore, the relationship between urine albumin excretion and increased renal or cardiovascular risk is continuous, and consideration of all ACR values under 30 mg/g as normal is likely inappropriate *(1)*.

Preanalytical Variables Affecting Albumin Excretion Rate

Table 2 lists important preexamination factors that may influence urine albumin excretion.

WITHIN-SUBJECT BIOLOGICAL VARIATION

Knowledge about the within-subject biological variation is important for making decisions about which types of samples should be used for urine albumin measurement, for interpreting a confirmatory result following an initial result showing an increased concentration, and for deciding whether or not a change in albumin excretion is of clinical importance.

Table S1 (in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol55/issue1) shows estimates of within-subject biological variability (CVi) for albumin excretion of 4%–103%, with a central tertile of 28%–47%. Factors that influenced the diversity in estimates include the period over which the samples were collected (days, weeks, months), the type of urine sample used (24 h, timed overnight, first morning, random), the design of the study, the concentration of urine albumin, the health condition of subjects, and the preanalytical handling and storage of the urine samples. Most of the studies did not describe how the CVi was calculated in sufficient detail to allow meaningful understanding of the differences.

Nevertheless, some general conclusions can be drawn from Table S1. The CVi of the ACR had the lowest value in 22 of 30 studies (73%), for which the CVi of 24-h excretion, overnight excretion rates, or concentrations were compared with the CVi of the ACR. The CVi of the ACR for day-to-day variation in predominately timed overnight and morning samples is in general lower than the ACR for week-to-week or month-to-month intervals. However, the variation in CVi from different studies is large and probably due to differences in the methods used (e.g., preanalytical factors such as storing samples before analyzing) and factors related to the calculations of the CVi (such as outlier exclusion and testing for homogeneity of variances). Omitting these last 2 factors tends to increase the reported CVi. Interestingly, the CVi for the ACR or albumin concentration has not been examined carefully in random urine samples, but there are studies indicating a higher CVi in these settings *(34, 35; S1 and S14 in the online Data Supplement)*. A second morning urine sample has been shown to be comparable to a 24-h sample *(36)*, and 2 studies listed in Table S1 in the online Data Supplement that used second morning samples gave CVi values comparable to other studies that used first morning samples. However, no direct comparison between first and second

Table 1. Summary of clinical practice recommendations for urine albumin measurement.[a]

Author/organization	Screening recommendations, including frequency, sample collection, testing methods	Reporting recommendations	Reference
American Diabetes Association[b]	Screening: Perform annual test for the presence of urine albumin in type 1 diabetic patients with diabetes duration of >5 years and in all type 2 diabetic patients, starting at diagnosis and during pregnancy. Methods: (*a*) Measurement of ACR in random spot collection (preferred method); (*b*) 24-h collection with creatinine, allowing simultaneous measurement of creatinine clearance; and (*c*) timed (e.g., 4-h or overnight) collection.	Not directly stated, but a table showing criteria for different diagnoses uses mg/g	*(2)*
International Diabetes Federation	Standard care: Check annually with dipstick for proteinuria in early morning urine sample (or random sample). Repeated/confirmation tests recommended, including ACR or PCR.[c] Comprehensive care: Assessment of albuminuria via laboratory quantitative method (ACR). Minimal care: Check annually with dipstick or sulfosalicylic acid method for proteinuria in early morning urine sample (or random sample otherwise). Repeated/confirmation tests recommended, including PCR.	Not directly stated, but standard of care section mentions mg/mmol and mg/g	*(3)*
Kidney Disease: Improving Global Outcomes	Preferred protein: Albumin is preferred urinary protein. Measurement: Albumin measurement procedures should be traceable to CRM 470 reference material. Multiple methods available. If necessary, antibody-based dipstick can be used; conventional dipstick in spot urine specimens acceptable if it is the only available option. Repeated/confirmation tests are recommended. Collection/sample: Random untimed spot urine samples are suitable for initial testing. First morning urine sample preferable, but not required if poses substantial inconvenience compared to random specimen. Timed urine collection for albumin and creatinine may be performed if reduced variability required.	mg/g	*(4)*
National Kidney Foundation (US)	Guidelines for adults and children: Under most circumstances, untimed spot urine samples should be used to detect and monitor proteinuria in children and adults. Usually not necessary to obtain a timed urine collection (overnight or 24-h) for evaluations. First morning specimens preferred. In most cases, screening with urine dipsticks is acceptable for detecting proteinuria. Standard urine dipsticks are acceptable for detecting increased total urine protein. Albumin-specific dipsticks are acceptable for detecting albuminuria. Repeated/confirmation tests are recommended, including PCR or ACR. Specific guidelines for adults: For screening of adults at increased risk for CKD, albumin should be measured in a spot urine sample by use of either albumin-specific dipstick or ACR.	Recommended units not identified	*(5)*
	Guidelines for diabetic patients: Patients with diabetes should be screened annually for CKD. Initial screening should commence 5 years after diagnosis of type 1 diabetes or from diagnosis of type 2 diabetes.Screening should include measurements of urinary ACR in spot urine sample. Repeated/confirmation tests are recommended.	mg/g	*(6)*

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Table 1. Summary of clinical practice recommendations for urine albumin measurement.[a] *(Continued from page 26)*

Author/organization	Screening recommendations, including frequency, sample collection, testing methods	Reporting recommendations	Reference
Caring for Australians with Renal Impairment	Testing for proteinuria: For high-risk populations initial test is PCR. For diabetes patients and aboriginals and Torres Strait Islanders: initial test is ACR. Repeated tests recommended. Method of assessment: Method choice should be tempered by practical considerations such as ease of use, patient acceptability, and cost. For initial testing for albuminuria, a first morning urine albumin sample is preferred; however, random ACR is acceptable. Repeated/confirmation tests are recommended; PCR is identified as an accurate test for diagnosis of significant proteinuria.	Table within guideline uses g/day, mg/dL, and mg/mmol for respective tests and diagnoses.	*(7)*
Joint Specialty Committee on Renal Medicine of the Royal College of Physicians of London and the Renal Association (UK)	Method for detection of urine albumin: Urine albumin should be measured by use of a laboratory (quantitative) method in early morning (preferred) or random midstream urine sample and expressed as ACR. If dipsticks designed to detect urinary albumin are used, positive tests should be followed by laboratory confirmation. Indications for testing: Patients with diabetes who have persistent proteinuria do not require testing for urine albumin. All other patients with diabetes should undergo, as a minimum, annual testing for albuminuria. Currently there is no proven role for screening for albuminuria in patients who do not have diabetes. Method for detection and quantification of proteinuria: No need to perform 24-h urine collections for quantification of proteinuria in primary care. Repeated/confirmation test is recommended, including ACR or PCR. Indications for testing: Dipstick urinalysis for protein is indicated as part of initial assessment of patients with newly discovered GFR <60 mL/min/1.73 m^2, newly discovered hematuria, and several other conditions. Do not recommend dipstick urinalysis screening for any other groups.	mg/mmol	*(8)*
UK Renal Association Clinical Practice Guidelines: Clinical Practice Guidelines for the Care of Patients with Chronic Kidney Disease	Patients being investigated or treated for CKD, proteinuria detected by dipstick testing should be assessed by measurement of either PCR or ACR, ideally on an early morning urine specimen.	No specific recommendation	*(9)*
National Institute for Clinical Excellence (UK)	Renal care for all people with type 2 diabetes: Measure ACR or albumin concentration annually. Use first morning urine sample where practicable; use laboratory or near-patient test specifically for microalbuminuria. Repeated/confirmation tests are recommended.	mg/mmol	*(10)*
National Kidney Foundation (US)	Guideline 2, Evaluation of Patients with Chronic Kidney Disease or Hypertension: ACR or PCR in first morning or random untimed spot urine specimen.	mg/g	*(11)*

[a] We used PubMed to locate published clinical guidelines or recommendations that include urine protein measurement as a part of the assessment of kidney disease or as a risk factor for cardiovascular disease. Searches included combinations of the terms urine albumin, urine protein, guidelines, measurement, kidney disease, and detection. The Kidney Disease Improving Global Outcomes website, which lists clinical practice guidelines from various organizations (http://www.kdigo.org/clinical_practice_guidelines/index.php; accessed November 2008) was also used as a source.

[b] Similar recommendations are found in the related guidelines of the National Academy of Clinical Biochemistry that were developed in cooperation with the American Diabetes Association (http://www.aacc.org/members/nacb/LMPG/Pages/default.aspx; accessed November 2008).

[c] PCR, protein/creatinine ratio; CKD, chronic kidney disease; GFR, glomerular filtration rate.

Table 2. Preexamination factors affecting urine albumin excretion.[a]

	Effect on urine albumin excretion	Consequences for urine albumin examination	References
Exercise	Increased	Should not be performed after hard physical exercise (evidence is not fully congruent)	*(12–22)*
Fever	Increased	Should not be performed until 3 days after fever	*(23–25)*
Asymptomatic bacteriuria	None	Screening for asymptomatic bacteriuria is not necessary.	*(26–28)*
Posture (orthostasis)	Increased	An increased urine albumin excretion in a young or adolescent person should be repeated by examination of a first morning urine sample.	*(29–33)*

[a] The findings are based on PubMed searches performed by using the text words "urine albumin" or "microalbuminuria" or "microalbumin" in combinations with "exercise" or "fever" or "bacteriuria" or "urine tract infection", respectively. "Proteinuria" was used in combination with "orthostatic" or "postural". For all searches, "Related articles" and the reference list of the retrieved articles were reviewed.

morning samples in the same study has been performed.

CHANGES IN URINE ALBUMIN DURING SAMPLE COLLECTION AND STORAGE

At low concentrations of albumin in urine, adsorption to the surface of storage containers can lead to significant losses. Binding of a monolayer of albumin to a surface requires about 0.15 $\mu g/cm^2$ *(37)*. Nonspecific adsorption of urinary albumin was calculated at <1 mg/L with hydrophilic surfaces and <2 mg/L with nonhydrophilic surfaces *(38)*. Binding to surfaces also results in some denaturation, and both adsorption and denaturation can be reduced when a suitable hydrophilic surface is used or nonionic detergent added *(39, 40)*. Albumin appears to be relatively stable at the air–liquid interface when foaming is generated by rapid mixing *(41)*.

There have been variable recommendations regarding the long-term storage and stability of urine samples for albumin analysis *(42–46)*. Recent findings suggest that urine samples are stable for long periods when frozen at −80 °C. Frozen storage at temperatures above −80 °C, particularly at −20 °C, has been reported to produce various modifications to albumin *(46–50)*.

For routine clinical laboratory testing, fresh urine collected from midstream is preferable. Albumin is generally stable in urine stored at 2–8 °C for 7 days *(36, 42)*. The influence of bacteria and proteases has not been well studied, but both may cause changes in albumin in some urine samples. Precipitates often form in refrigerated or frozen urine, and their effect on albumin measurement has not been thoroughly investigated. Precipitates frequently redissolve when the urine is warmed for analysis. Thawing frozen urine at 37 °C has been reported to minimize precipitate *(51)* but could increase the rate of protease activity. Centrifugation of cloudy urine may be needed to remove insoluble material before measurement.

URINE SAMPLE COLLECTION AND TESTING PRACTICES

Surveys of clinical practice have shown a wide range in sample collection and choice of measurement procedures and decision thresholds used. A study from 2002 described urine albumin testing practices in hospitals and physician offices in Montana (USA) and found that only 43% and 46%, respectively, used American Diabetes Association or National Kidney Foundation (USA) recommended thresholds *(52)*. A French study in 2003 of general practitioners caring for diabetic patients reported that only 36% of patients had a urine albumin test and that only a 24-h urine collection was considered adequate *(53)*. A survey of British pediatric diabetes care practices reported in 2005 that ACR measurement in morning samples was the predominant test (81%), whereas timed overnight collections and 24-h collections were used by 14% and 5%, respectively *(54)*.

During 2006, a questionnaire-based evaluation of urine albumin assessment in primary healthcare was conducted in 9 European countries, with replies received from 2078 general practitioners *(55)*. Spot urine samples prevailed for first-time examination of urine albumin, whereas timed collections were used to a larger extent for a repeat test, especially when carried out in a hospital laboratory. Only 45%–77% of general practitioners requested a repeat test if the first test was positive. Prevalence of office-based instruments for urine albumin analysis varied from 4% to 88% among countries, with highest numbers from Norway and Sweden, and the lowest in France. When office instruments were used, quantitative ACR measurements prevailed in Scandinavia, whereas semiquantitative albumin strips were in widespread use in the other countries. In all countries but the Netherlands, 4 dif-

ferent ways of reporting urine albumin results were used: concentration (mg/L), excretion per 24 h (mg/24 h), excretion per minute (μg/min), and ACR (mg/mmol or mg/g). Physicians in most countries estimated that a 33% change in urine albumin results indicated a clinically significant change in a patient's condition independent of the type of sample that was used for the measurement.

In 2006, an Australia/New Zealand practice survey of 55 laboratories showed marked variability in the recommended sample types and sample collection, including 24-h urine collection, other timed samples, random spot sample, and collection of the first morning urine specimen *(56)*. The decision limit reported by laboratories varied from 15 to 30 mg/L for urine albumin and 1.0 to 3.6 mg/mmol (9–32 mg/g) for the ACR. There was no relationship between the reference intervals and the instrument/method used.

Molecular Forms of Albumin in Urine

The abundance and molecular forms of albumin in urine may differ from those in plasma because of differential filtration or tubular uptake of modified forms of albumin, modification of albumin by proteolysis during passage through the urinary tract; chemical modification by oxidants, free radicals, and other ligands concentrated in urine; and modification during specimen storage.

ALBUMIN STRUCTURE

Structural properties of plasma albumin have been reviewed by Peters *(57)*. The gene for human serum albumin encodes a precursor, preproalbumin, which is processed intracellularly to the mature plasma protein of 585 amino acid residues secreted by hepatocytes. Albumin's polypeptide does not undergo posttranslational modifications intracellularly. A high content of acidic amino acids contributes to a net charge of −15 to −20 at neutral pH, an isoelectric point near 5, and high aqueous solubility. X-ray crystallography shows a heart-shaped protein, with 3 globular domains forming a V *(58, 59)*. Albumin is stabilized by 17 internal disulfide cross-links and a high content of α-helical structure, resulting in a molecule that is relatively resistant to denaturation *(57)*. Albumin in solution behaves hydrodynamically as a 14-nm–long cylinder. The elongated shape and increased hydrodynamic size may be important for decreasing glomerular filtration of albumin.

Mutant alleles of albumin are expressed in <1 of 1000 people *(57)* and thus should rarely affect quantitative analysis of albumin. Some point mutations, however, affect renal albumin clearance *(60)*, producing different proportions of a modified albumin in urine than in serum.

The usual pH of urine (pH 5–8) does not affect the shape of albumin. Below pH 4 and above pH 8, albumin undergoes major conformational changes, most of which are reversible *(57)*. The maximum concentration of urea in urine is about 1 mol/L and should not cause denaturation *(57)*.

Albumin has up to 6 binding sites per molecule for long-chain fatty acids. In plasma, there is usually about 1 fatty acid per albumin molecule, but this ratio can increase several-fold with stress, exercise, or heparin therapy. Variable amounts of bound fatty acids alter the electrophoretic mobility of albumin in nondenaturing electrophoresis and isoelectric focusing *(61, 62)*.

Albumin is a carrier for numerous small organic molecules and ions *(57, 63–68)*. Binding of these molecules could affect conformation of albumin *(57, 66, 69)*. A number of other endogenous compounds, such as copper and thyroxine, bind to albumin but occupy <1% of albumin molecules in plasma. Bilirubin occupies a small proportion of albumin molecules, except in severely hyperbilirubinemic states, in which more than half of albumin molecules may have bilirubin ligands. At physiological concentrations in plasma, 1–2 calcium ions and 7–8 chloride ions are bound per albumin molecule *(67–68)*. Binding of these ions is pH dependent and thus variable in urine, and ions might rapidly dissociate or exchange with other ions during analysis.

Urine is enriched relative to plasma in low molecular weight peptide components and amino acid derivatives such as hippuric acid and phenylacetylglutamine *(47, 70, 71)*. On a molar basis, the urinary concentrations of these compounds usually exceed that of albumin, so there may be substantial ligand binding to albumin *(70, 71)*. In plasma, albumin also binds a diverse range of peptides for which there are limited data regarding affinity and stoichiometry of binding *(72, 73)*.

Albumin contains 2 sites (Sudlow sites I and II) for binding of numerous drugs and endogenous compounds *(63)*. Some of these compounds, such as salicylates, sulfa antibiotics, and penicillins, occur in high concentrations in urine.

COVALENTLY MODIFIED FORMS OF ALBUMIN

Albumin contains an unpaired cysteine at residue 34. It has an unusually low pK of about 5 that results in increased reactivity and rates of disulfide-bond formation and exchange with other sulfhydryl-containing compounds in plasma *(57, 74, 75)*. Albumin can form disulfide-linked dimers, with 2 albumin monomers

linked by cysteine-34 residues, or the cysteine side-chain can be oxidized to a sulfonic acid *(57, 76)*. Albumin with various modifications of cysteine-34 has different binding properties for a variety of ligands, suggesting a significant conformational change *(57, 77)*. Some albumin dimers, primarily disulfide linked, have been observed in urine specimens *(47, 78–82)*.

Albumin has a long half-life of about 20 days in circulating blood *(57, 83)*, which allows albumin to accumulate chemical modifications. Reactions with amino acid side-chains yield carbonyl group, carboxymethyllysine, and advanced glycation end products on a small proportion of albumin molecules *(76, 84, 85)*. Dityrosine cross-links also have been detected *(77)*. In plasma 1%–10% of albumin molecules are glycated by reaction with glucose, with increased concentrations in people with diabetes *(57, 86)*. The higher proportion of glycated albumin in urine than plasma has been attributed to lower efficiency of tubular uptake of the glycated form *(86)*. Tubular uptake of albumin is a receptor-mediated process with considerable specificity *(87, 88)*.

FRAGMENTATION OF ALBUMIN

Several albumin fragments >5 kDa have been detected in urine *(47, 78–81, 89–94)*. The proportions of fragments have been observed to increase with kidney disease *(78–81)* and possibly with prolonged storage of specimens at −20 °C *(47)*. The sequences of several large fragments in urine have been identified, and some were detected in plasma, suggesting that some fragments in urine were from plasma *(81)*.

Albumin fragments of 500–5000 Da have been reported in plasma and urine *(95–103)*. Some accumulate in plasma of patients with renal failure. Glomerular filtration is a size- and charge-dependent process, and small positively charged fragments generated in the plasma should be rapidly cleared by the healthy kidney *(104, 105)*.

Several truncated forms of albumin have been detected in plasma, with deletions of 1 or 2 N-terminal amino acids *(106)* or 1, 6, or 13 C-terminal amino acids *(107, 108)*. Albumin lacking the C-terminal leucine residue constitutes 4%–15% of albumin from normal plasma *(107)*, and it can become the major form of albumin in plasma and urine of patients with critical illnesses *(107, 108)*.

Proteases along the urinary tract and in urine may generate albumin fragments or further modification of fragments in the urinary tract and during specimen storage *(109–111)*. Some albumin taken into tubular cells may be partially digested and the fragments returned to the urine *(112)*.

INFLUENCE OF URINE ALBUMIN STRUCTURAL FORMS ON MEASUREMENT

Immunoassay has been the primary method for quantifying urine albumin. Human albumin is highly antigenic in many animal species *(57)*. The polyclonal response of rabbits is directed against at least 5 different antigenic sites *(113)*, suggesting that immunoassays with polyclonal antisera can react with many modified albumins. The existence of several antigenic sites is consistent with evidence that an immunoturbidimetric assay reacted with albumin cleaved into 3 pieces by cyanogen bromide, with chemically modified albumin and with animal albumins that differed in amino acid sequence by more than 20% from human albumin *(47)*.

Current Routine Methods for Measuring Urine Albumin

ROUTINE MEASUREMENT PROCEDURES FOR URINE ALBUMIN

Urinary concentrations of albumin <150 mg/L are below the detection limit of the colorimetric test strips ("dipstick" tests) used in routine urinalysis. Available immunoassays, including turbidimetric, nephelometric, and 2-site immunometric procedures typically have limits of detection of 2–10 mg/L *(114, 115)*. Formats include liquid reagents with quantitative nephelometric or spectrophotometric measurement and lateral flow strips with semiquantitative visual determination. Routine clinical methods use both polyclonal and monoclonal antibodies that may influence their sensitivity to measure altered forms and fragments of albumin.

Size-exclusion liquid chromatography has been applied as an alternative method and gives higher values than immunoassay for most specimens. This observation has lead to a controversial hypothesis that size-exclusion chromatography detects a form of albumin that is not detected by immunoassay *(114, 116–118)*. This hypothesis has been questioned on the basis of the documented reactivity of polyclonal antisera with multiple antigenic sites in albumin *(82, 113)*. Moreover, the results of the size-exclusion method included other molecules of approximately the same size as albumin, including several urinary proteins *(82)*.

PERFORMANCE OF MEASUREMENT PROCEDURES FOR URINE ALBUMIN

There are no data reported on the uniformity of results between methods and between laboratories using freshly collected samples. Consequently, we examined between-laboratory and between-method variation in results from external quality assessment schemes (EQAS). In principle, the samples used in such surveys should reflect the content and composition of albumin

in native urine and be commutable with native urine. In practice, the urine samples used in EQAS are frequently prepared with added purified albumin and creatinine, and may include other analytes, stabilizers, and pH-adjustment additives. Such samples may have a less complex matrix and a more homogeneous albumin molecule than found in native urine and give EQAS results that are more uniform than might be seen with native urine.

Table 3 demonstrates that different EQAS organizers use different materials and treat them in different ways. Samples that use liquid native urine with limited or no addition of purified albumin and creatinine are more likely to be commutable. NOKLUS (the Norwegian Center for Quality Assurance in Primary Health Care) experience indicates that urine samples from patients with albuminuria behave differently with some methods compared to normal urine with added purified human albumin. The more synthetic and the more processed a sample, the more uncertain its commutability. Samples that have been lyophilized are not likely to be commutable with native samples. Noncommutable samples are limited to evaluating agreement of results within a method/instrument peer group and cannot be used to evaluate agreement between different methods.

Table 4 presents examples of the range of results observed from several EQAS programs in different countries. When the EQAS material was considered to have a reasonable likelihood to be commutable, the data for all participants were combined to represent aggregate between-method and between-laboratory performance. When the material was less likely to be commutable, the data were separated by methods to give a between-laboratory within-method estimate of performance. All EQAS exclude outliers (using different procedures) before calculating statistics. The ± 2 SD range was calculated from available data to provide an estimate of a central 95% range of reported results.

All surveys demonstrated variability among laboratories and among measurement procedures (Table 4). The between-laboratory within-method variation was smaller than the combined between-laboratory between-method variation, suggesting there were calibration differences among methods. It is difficult to evaluate whether current analytical performance meets clinical requirements because those requirements have not been defined on the basis of outcomes evidence. If a quality specification for imprecision of urine albumin concentration measurement is one-half of the intraindividual biological variation and a typical CVi is taken as 40% (Table S1 in the online Data Supplement), the EQAS results can fulfill this criterion. Bias cannot be evaluated because there is no reference system in place.

A Reference System for Urine Albumin Measurement

A reference system for urine albumin requires both a primary and secondary (matrix) reference material (RM) and a reference measurement procedure (RMP) with which the assigned value of an RM can be accurately transferred to a patient sample through the measurement hierarchies of the traceability chain *(119)*. At this time, the Joint Committee for Traceability in Laboratory Medicine (JCTLM) web site does not list a higher-order RM or RMP for urine albumin *(120)*.

An RM intended for calibration of routine measurement procedures should be commutable with native urine for all procedures. This attribute means that the routine methods will have equivalent immunochemical reactivity toward the albumin molecule(s) in the RM as for albumin in native urine samples. Commutability is more difficult to define for urine because the matrix is highly variable in different pathologic conditions and the measurand is not well defined. A reference material that uses highly purified albumin may not reflect the various molecular forms present in typical urine. However, use of purified albumin in an RM may be the most practical approach to achieve standardized calibration of routine measurement procedures.

MATERIALS AND METHODS CURRENTLY USED AS REFERENCE FOR CALIBRATION OF URINE ALBUMIN MEASUREMENT PROCEDURES

Because no urine albumin RM currently exists, most routine methods are calibrated to be traceable to diluted CRM470 (now called ERM-DA470; Institute for Reference Materials and Measurements, Geel, Belgium), a higher-order serum protein RM with an albumin concentration of 39.7 g/L *(121)*. No agreed dilution protocols for preparing concentrations of CRM470 suitable for urine method calibration have been published. The concentration of albumin in CRM470 was originally assigned based on an older serum protein RM, USNRP 12–0575C *(122)*. However, the protein structure, physicochemical properties, and value-assignment procedure for USNRP 12–0575C have not been elucidated *(121)*. An IFCC Working Group has attempted to design a similar reference material for urinary proteins including albumin but has not accomplished this goal *(123)*.

Japanese investigators reported that 5%–10% of the calibrators used with routine immunoassays contained polymerized albumin *(51)*. The same study revealed that some methods used diluted CRM470 as the basis for calibrator value assignment whereas others used the molar absorption coefficient of human serum albumin.

Table 3. Overview of EQAS programs from several countries, showing materials used for urine-albumin surveys.

EQAS program[a]	Type of sample	Conditions during sample preparation	Conditions before circulation to participants	Conditions during circulation to participants	Likely to be commutable[b]
EQUALIS (Sweden)	Normal urine	Benzamidinium chloride added to normal urine. Frozen, thawed and filtered. Albumin added and refrozen at −80 °C¤	Thawed and diluted with NaCl and creatinine added	Liquid at room temperature	Yes
Labquality (Finland, Norway, and others)	Normal urine	Fresh urine	Supplemented with albumin and creatinine	Liquid at room temperature	Yes
NOKLUS (Norway, GPs)	Urine from patients with albuminuria	Frozen at −80 °C	Thawed and sterile filtered	Liquid at room temperature	Yes
QMP-LS (Ontario, Canada)	From single donor patient with albuminuria	Second sample from another patient with albuminuria may be added to adjust concentration	Stored at 4 °C	Liquid and shipped with cold pack.	Yes
Digital PT (British Columbia, Canada)	Stabilized synthetic (not donor) urine	Liquid at 4 °C	Liquid at 4 °C	Liquid and shipped with cold pack.	Unknown
CAP (USA)	Normal urine	Added albumin, creatinine, other analytes, preservatives	Stored at 4 °C	Liquid and shipped with cold pack.	Unknown
RCPA-QAP (Australia)	Normal urine	Added albumin, creatinine, other substances and lyophilized	Nothing after lyophilization, stored at 4 °C	Lyophilized	No

[a] EQUALIS, External Quality Assurance in Laboratory Medicine in Sweden; NOKLUS, Norwegian Quality Improvement of Primary Care Laboratories; GP, general practitioner; QMP-LS, Quality Management Programme, Laboratory Services; CAP, College of American Pathologists; RCPA-QAP, Royal College of Pathologists of Australasia Quality Assurance Programs.

[b] The commutability to fresh native urine from patients with increased excretion of albumin is based on theoretical considerations of EQAS sample preparation; commutability has not been validated for any materials.

Table 4. Variation of urine albumin results observed in EQAS surveys from different countries.[a]

EQAS program[b]	N	Median or mean, mg/L	CV, %	±2 SD range
EQUALIS	200	20	10.8	16–25
EQUALIS	200	31	8.2	26–37
Labquality	136	19	15.4	14–25
Labquality	136	76	8.9	63–89
QMP-LS	28	20	16.5	14–26
QMP-LS	29	22	10.9	17–26
QMP-LS	28	58	6.9	50–66
NOKLUS (GP's offices)	1012	35	12.1	27–44
NOKLUS turbidimetry	430	20	6.6	17–44
NOKLUS turbidimetry	424	68	5.2	61–74
Digital PT	38	36	11.3	28–44
Digital PT	37	74	8.4	61–86
CAP method A, turbidimetry	262	25	10.4	20–27
CAP method A, turbidimetry	207	87	5.1	79–92
CAP method B, turbidimetry	203	27	6.9	23–29
CAP method B, turbidimetry	194	83	3.1	78–85
CAP method C, turbidimetry	162	30	7.1	26–32
CAP method C, turbidimetry	123	88	4.5	80–92
CAP method D, turbidimetry	118	26	5.0	24–28
CAP method D, turbidimetry	86	84	3.8	78–87
CAP method E, turbidimetry	76	24	6.2	21–25
CAP method E, turbidimetry	112	87	4.9	78–91
CAP method F, turbidimetry	96	26	10.1	21–29
CAP method F, turbidimetry	82	89	4.9	81–94
CAP method G, nephelometry	95	27	6.2	23–28
CAP method G, nephelometry	79	86	5.3	76–90
CAP method H, nephelometry	97	26	7	23–28
CAP method H, nephelometry	69	93	4.5	84–97
RCPA method A, turbidimetry	30	36	8.5	30–42
RCPA method A, turbidimetry	30	83	5.8	73–93
RCPA method B, nephelometry	20	35	10.5	28–42
RCPA method B, nephelometry	20	85	6.2	74–95

[a] When an instrument method is given, the data reflects the between-laboratory within-method variation; otherwise the combined between laboratory and method variation is given.
[b] Abbreviations as in Table 3.

CANDIDATE REFERENCE MATERIAL FOR URINE ALBUMIN

The Japanese Society of Clinical Chemistry and the Japanese Committee for Clinical Laboratory Standards have coordinated development of a new urine albumin candidate secondary RM with well-defined protein structure and physicochemical properties *(124)*. The RM was prepared using >97.5% pure monomeric human serum albumin in a matrix of 0.5 mol/L NaCl, 20 g/L sucrose, and 0.5 g/L NaN_3 in 20 mmol/L phosphate buffer, pH 7.3. The lyophilized material had an intervial difference <3%, and was stable >1 year at 5–10 °C and 20 h after reconstitution with water at both 10 °C and 25 °C.

Because there is no RMP available for urine albumin, the candidate RM was value-assigned with traceability to diluted CRM470 by use of routine immunoassay procedures *(124)*. In summary, 13 participating routine measurement systems had identical immuno-

chemical reactions with the candidate RM and diluted CRM470. The concentrations determined for the candidate RM by each routine method's value transfer from CRM470 were averaged to assign a concentration for the candidate RM of 226.1 (8.4) mg/L [mean (uncertainty)] after reconstitution with 3 mL of water.

The Japanese investigators plan to validate commutability for the new candidate RM and submit it to JCTLM for approval. They are also investigating use of recombinant human albumin to develop a primary reference material based on the technology used for the candidate secondary RM.

CANDIDATE REFERENCE MEASUREMENT PROCEDURE FOR URINE ALBUMIN

An RMP for urine albumin should specifically measure the albumin molecule(s) in native urine. Because of the heterogeneity of molecular forms of albumin in urine, the measurand is not clear and must be defined. Immunological procedures are not suitable as RMPs because antibodies in different procedures may react with different epitopes on the albumin molecule or molecular fragments and different measurement principles may give different responses.

Researchers at Mayo Clinic Rochester recently developed an LC-MS measurement procedure that measures the N-terminal 24–amino-acid fragment of albumin. The method employs source-induced fragmentation, designed to obviate the need for trypsin digestion and potential issues with incomplete digestion. Similar results were obtained using either ^{15}N-labeled human serum albumin prepared in a yeast expression system *(125)* or less expensive bovine serum albumin *(126)* as internal standards. Calibration was based on a charcoal-stripped urine supplemented with commercial human serum albumin whose concentration was quantified by the molar absorptivity [38553 L/(mol-cm)] at 280 nm *(127)*.

Because the procedure quantifies a specific urine albumin fragment, it will be necessary to investigate to what extent normal and diseased urine contains albumin fragments with these 24 amino acids or N-terminal truncated albumin molecules. Use of LC-MS to detect other fragments of albumin could facilitate study of the nature, quantity, and clinical relevance of urinary albumin species in kidney and cardiovascular diseases. LC-MS technology is a nonantibody-based technique that may be developed into a candidate RMP for urine albumin.

A REFERENCE SYSTEM FOR URINE CREATININE MEASUREMENTS

The determination of ACR relies on measurement of both albumin and creatinine in urine. The variability of calculated ACR values will reflect the combined bias and imprecision of measurement of both analytes. Standardization for both urine albumin and urine creatinine measurements is necessary to obtain comparability of ACR values obtained with different methods and in different locations.

The NKDEP-initiated standardization program for serum creatinine was based on the existence of validated reference measurement procedures and development of a commutable secondary RM for serum creatinine. A similar effort is needed to promote high-quality routine measurements of creatinine in urine. The JCTLM has listed a reference measurement procedure for urine creatinine based on isotope dilution–gas chromatography–mass spectrometry *(128)*, and a certified primary (i.e., pure substance) RM for creatinine exists (National Institute for Standards and Technology, USA, SRM 914a). However, no secondary matrix-based RMs are currently available for urine creatinine.

Because of the lack of certified secondary RMs for urine creatinine, calibration of routine methods of urine measurement is often performed with serum-based RMs. This practice is not ideal, however, because of the important differences between the matrices of urine and serum.

Conclusions

A number of issues require clarification to improve use of urine albumin for assessment of kidney disease. Existing information does not support strong conclusions or practice recommendations at this time. Additional investigations are needed to obtain information and objective evidence to enable standardization of measurement of urine albumin and clinical practice guidelines based on measurement of urinary excretion of albumin. The NKDEP/IFCC group intends to develop experimental programs to acquire the additional information.

CURRENT PRACTICES THAT REFLECT THE CONSENSUS OPINIONS OF THE CONFERENCE PARTICIPANTS

1. Use of the term "urine albumin" is recommended; "microalbumin" is discouraged.
2. First morning void urine samples provide lower variability than random samples.
3. Second morning void urine samples may also be acceptable but there is no evidence to support this practice as superior to first morning void.
4. Urine albumin should be measured in urine that has not been frozen. Albumin in urine is adequately stable when stored at 2–8 °C for 7 days before measurement. Any cloudiness due to precipitate or cellular components should be removed by centrifugation before refrigerated storage.

5. Refrigerated urine should be warmed to room temperature before measurement to dissolve precipitates that may have formed and adsorbed albumin. The urine should be visually examined for precipitate and centrifuged if necessary to remove residual precipitate.
6. If urine is to be frozen before measurement, it should be frozen at ≤–70 °C. Any cloudiness due to precipitate or cellular components should be removed by centrifugation before frozen storage. Thawed samples should be warmed to room temperature and mixed thoroughly before measurement. The effect of freezing and thawing on albumin molecular forms is not thoroughly understood.
7. An albumin/creatinine ratio should be reported with all urine albumin measurements.
8. Confusion arises from reporting results in units of "mg albumin/mmol creatinine," "g albumin/mol creatinine," "mg albumin/g creatinine," or "μg albumin/mg creatinine." This situation reflects national or regional preferences and is not likely to be resolved. Ideally, International System of Units should be adopted. In the interim, uniform guidelines should be followed within a country or region.
9. Albumin concentrations reported in milligrams per liter are difficult to interpret and concentrations in these units should not be the only value reported.

ISSUES REQUIRING FURTHER INVESTIGATION FOR STANDARDIZATION OF MEASUREMENT AND REPORTING OF URINE ALBUMIN

1. Clarification of preanalytical requirements.
   - Influence of container type.
   - Influence of timing of collection (first morning, second morning, random, 24 h) related to biological variability.
   - Influence of blood (menstrual or urinary bleeding), seminal fluid, and other physiologic contaminants of urine.
2. Clarification of the molecular forms of albumin in freshly voided urine, and the definition of the measurand.
3. Clarification of the degree of urine albumin degradation under various conditions of storage.
4. Clarification regarding the variation in urinary matrix composition over which urine albumin measurement procedures must operate.
5. Clarification of the clinical requirements for total error in measurement of urine albumin.
6. Development of a reference measurement procedure.
7. Development of a urine albumin secondary reference material including its commutability validation and credentialing by JCTLM.
8. Development of a urine creatinine secondary reference material including its commutability validation and credentialing by JCTLM.
9. Identification of appropriate EQAS materials that will allow performance of routine methods to be compared.
10. Standardized measurement results are necessary to enable clinical studies to determine the optimal decision thresholds for AER and ACR.
11. Different decision limits may be needed for random vs first morning or other standardized collection time, owing to the increased variability of randomly collected samples.
12. The ACR varies with age, sex, and ethnicity. Decision thresholds suitable for these subgroups need further investigation. A single decision threshold may not be adequately sensitive for each subgroup.
13. Risk of chronic kidney disease and cardiovascular disease are continuous functions of urine albumin concentration. The appropriate thresholds for risk for given populations (e.g., general population or high risk groups such as diabetes, hypertension or cardiovascular disease) need to be determined.
14. Investigation of the usefulness of age- and sex-specific equations to convert ACR to an estimated AER for which a single reference limit may be appropriate.

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