Mini-Review

Laboratory Measurement of Urine Albumin and Urine Total Protein in Screening for Proteinuria in Chronic Kidney Disease

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Abstract
Laboratory measurement of urine total protein has been important for the diagnosis and monitoring of renal disease for decades, and since the late 1990s, urine albumin has been measured to determine whether a diabetic patient has incipient nephropathy. Evolving understanding of chronic kidney disease (CKD) and, in particular, the cardiovascular risks that CKD confers, demands more sensitive detection of protein in urine. As well, evidence is now emerging that cardiovascular and all-cause mortality risks are increased at levels within the current 'normal' range for urine albumin. Standardisation is essential to permit valid application of universal decision points, and a National Kidney Disease Education Program/International Federation of Clinical Chemistry and Laboratory Medicine (NKDEP/IFCC) Working Party is making progress towards a reference system for urine albumin. In the meantime, available data suggest that Australasian laboratory performance is adequate in terms of precision and accuracy above current decision limits for urine albumin. In contrast, the complexity of proteins in urine makes standardisation of urine total protein measurement impossible. As well, urine total protein measurement is insufficiently sensitive to detect clinically important concentrations of urine albumin. An Australasian Expert Group, the Proteinuria Albuminuria Working Group (PAWG) has proposed that urine albumin/creatinine ratio is measured in a fresh, first morning, spot sample to screen for proteinuria in CKD. Both NKDEP/IFCC and PAWG emphasise the need for standardisation of sample collection and handling.

Standardisation of Urine Albumin Measurement

Standardisation of urine albumin is required because currently common decision limits are used and it is likely that misdiagnosis occurs as a result of differences in calibration. As well, evidence suggests that risks are continuous variables commencing at levels below the current lower limit for microalbuminuria.[1] Standardisation requires a primary reference system comprising a defined measurand, measured against an appropriate reference material that is value assigned by a reference measurement procedure and, to date, none of these components has been confirmed. In 2007, the National Kidney Education Program (NKDEP) and the IFCC formed a joint committee whose objective is to co-ordinate the creation of such a system and to standardise the laboratory components such as sample collection and result reporting.[2]

A candidate reference method for urine albumin has been developed by the Mayo Clinic that uses the 24 amino acid fragment from the N-terminus of bovine serum albumin as an internal standard in a liquid chromatography-mass spectrometry measurement system.[3] Studies to validate the candidate method as a reference measurement procedure for intact albumin and to compare sensitivity and specificity with routine immunoassays are being undertaken in collaboration with the National Institute of Standards and Technology (NIST). There are plans to submit the method to the Joint Committee for Traceability in Laboratory Medicine (JCTLM) and it is anticipated that full credentialing will occur within the next several years.

The Japanese Society for Clinical Chemistry, in conjunction with the Japanese Committee for Clinical Laboratory Standards, has produced a candidate secondary reference material that is >97.5% pure monomeric human albumin and it has plans to submit this material to the JCTLM. Preliminary studies have shown identical immunoreactivity of this

material and the human plasma proteins reference material, CRM470, in 13 routine measurement systems.[4] Currently a few routine methods use the molar absorption coefficient of albumin in water as a basis for calibration. However, most methods use CRM470 (current lot DA470k/IFCC) as their calibration anchor. Despite using the same reference material, transferability may be limited by the use of different preparation and value transfer protocols.

Albumin Species in Urine

One of the difficulties in defining the measurand is that the composition of albumin molecules in urine is complex and varies significantly, even between healthy individuals. Native albumin in serum is a 585 amino acid protein of molecular mass 66,473 Da. It has 17 disulfide bonds, 4 globular domains and binds to multiple ligands including fatty acids, bilirubin, calcium and magnesium; there are both N- and C-terminal truncations, and significant glycation (1-10%, higher in diabetes). Conformation changes in plasma may influence the filtration rate at the glomerulus, while tubular uptake is receptor-mediated. Albumin in urine is exposed to a wider range of pH and ionic strength than found in plasma; other potentially modifying factors include the presence of high concentrations of urea, glucose and ascorbate, and cleavage by peptidases.[5]

Measurement of Urine Albumin

As in other parts of the world, routine measurement procedures for urine albumin in Australasia are predominantly (94%) immunoassay-based, mostly (85%) immunoturbidimetric. Work done by Comper and colleagues[6] in 2002 suggested that immunoassay methods might significantly underestimate albumin, particularly in diabetic patients. However, subsequent work questioned the definitive resolution of albumin by the HPLC method used.[7] Not all immunoassays perform equally and whether modified albumin is measured appears to depend on assay design, in particular whether polyclonal or monoclonal antibodies are used. Albumin has at least five antigenic sites and studies using polyclonal antibodies in a competitive assay design have been shown to measure some modified forms as well as non-human forms of albumin.[8]

While standardisation is clearly important, the major cause of variation in urine albumin measurement occurs outside the analytical process and much can be done to improve practice in both the pre- and post-analytical phases. Important pre-analytical factors are listed in Table 1; the post-analytical phase is beyond the scope of this article.

Specimen Handling

Albumin is stable in untreated urine for at least one week when stored at either 4 or 20 °C.[9] Freezing at -20 °C causes fragmentation, and a single freeze-thaw cycle may cause apparent albumin loss of 40% although the effect varies with storage duration and between individual samples.[10] Samples should ideally be analysed fresh. If prolonged storage is required, freezing at -80 °C appears to preserve albumin integrity when measured by immunoassay.[11] Creatinine is also stable in urine for at least one week at either 4 or 20 °C and is unaffected by freezing at either -20 or -80 °C. Prior to analysis, urine should be inspected for clarity and centrifuged if visibly cloudy.

Recommended Collection Type

Traditionally, albumin excretion rate (AER) was measured on a 24-hour urine collection and most data monitoring response to therapy has been obtained on such collections. This led to the perception by many clinicians that these collections represent the gold standard despite being inconvenient to the patient and notoriously unreliably collected. Timed, usually overnight, collections were introduced to reduce patient burden. However, there is no evidence that timed collections provide more clinically valid information than a urine albumin/creatinine ratio. Hence major international guidelines including those of the American Diabetes Association, Caring for Australians with Renal Impairment, International Diabetes Federation, National Institute for Clinical Excellence (UK) and National Kidney Foundation (US) now recommend measuring albumin/creatinine ratio (ACR) on a spot sample.

Surveys conducted in 2006 and 2009 show that the majority of samples presented to Australasian laboratories for urine albumin measurement are random, spot collections regardless of the laboratory's collection recommendation.[12]

Collection Container

There is some evidence that albumin binds to the surface of some plastics which may lead to under-recovery of albumin at low concentrations.[13] Studies are being conducted at the Centre for Disease Control under the auspices of the NKDEP/IFCC Working Party to quantify this effect. In the meantime, it seems prudent to collect an adequate volume to minimise potential losses.

Biological Variation

Available data report wide within-individual variation (CVi) in all urine albumin collection types with common estimates of 28-47%. Most studies confirm a lower CVi for ACR compared with AER, and for first void ACR or overnight AER compared with daytime collections.[2] In a healthy individual, postural or exercise effects can cause a potentially misleading elevation in ACR/AER and so many expert groups, including NKDEP/IFCC and PAWG recommend a first morning spot collection for screening purposes.

Table 1. Pre-analytical factors affecting urine albumin concentration.

Patient	Sample
Hydration status	Collection type
Exercise	Adsorption to plastic
Fever	Storage temperature
Posture	Sample clarity

Analytical Requirements

Individual variation informs test performance requirements and clinical interpretation. A wide CVi reduces the analytical performance requirements for imprecision (CVi/2), and in the most recent Royal College of Pathologists of Australasia Quality Assurance Program (RCPA QAP) end-of-cycle report (EOCR) for urine albumin, all Australasian laboratories achieved a CV <15% (Table 2). Moreover, 92% of participating laboratories achieved a CV < 10%, a performance goal newly recommended by PAWG. In the absence of standardisation, bias is more difficult to assess. Again, looking at data from the most recent EOCR, all 184 participants met the allowable limits of performance (ALP) criteria of +/- 4.0 mg/L at levels below 20 mg/L and +/- 20% above 20 mg/L, and a majority performed significantly better; 179 participants had a range of 5.7-10.5 mg/L for a target of 8.1 mg/L and 112.6-143.3 mg/L for a target of 132 mg/L (Table 2). Furthermore, a study reported by Tate and colleagues using fresh patient samples suggests results may be comparable across some methods, at least at levels greater than current cut-points.[14] The apparent

Table 2. Instrument Summary Data. End of Cycle Report for Urine albumin. RCPA QAP Basic Urine Chemistry Programme, Cycle 52 July – November 2010. Statistics are derived from 12 samples (duplicates at six concentrations ranging from Low to High) for each instrument group. SD and CV indicate the median imprecision for the 12 results, and results for Low and High indicate the median bias against the targets given. See the RCPA Chemical Pathology QAP website[18] for more detailed explanation of the statistical processes.

Instrument	No. Labs	SD	CV (%)	Low 8.1	High 132.0
Beckman Coulter LX20/LX40	1	1.24	1.8	8.4	130.7
Roche Diagnostics Hitachi Cobas c501/c502	22	1.85	2.7	9.7	129.2
Siemens Healthcare Diagnostics	1	2.20	2.8	8.1	151.6
Roche Diagnostics Hitachi Modular	15	2.30	3.2	10.5	132.5
INTEGRA 400/400+	7	2.23	3.2	7.7	132.0
ADVIA 1650/1800	5	2.49	3.4	5.7	148.9
Roche Diagnostics Hitachi 912	1	2.46	3.5	7.0	132.4
Abbott ARCHITECT c8000	11	2.63	3.5	7.5	143.3
Beckman Coulter AU2700/AU5421/AU5432	7	2.25	3.6	7.6	117.9
BN ProSpec	1	2.87	3.7	8.9	144.6
Beckman Coulter UniCel DxC 600/601i	13	2.75	3.7	8.2	140.5
Beckman coulter AU400	1	2.31	3.8	5.7	117.1
ADVIA 2400	13	2.97	4.0	6.1	140.8
Beckman Coulter AU600/640	9	2.63	4.1	7.7	118.6
Abbott ARCHITECT c4000/c16000	5	3.13	4.2	8.4	138.6
Roche Diagnostics Hitachi 917	2	2.97	4.2	10.1	129.9
Beckman Coulter Immage/Immage 800	10	2.75	4.4	6.8	121.8
Roche Diagnostics Hitachi Cobas c701/c702	1	3.6	5.1	11.0	133.4
Beckman Coulter UniCel DxC 800	7	4.05	5.7	8.6	138.0
INTEGRA 700/800	23	4.08	5.9	5.8	132.3
DCA Vantage	2	4.38	6.2	4.9	133.9
Siemens Healthcare Diagnostics DCA 2000	2	4.32	6.4	6.0	128.6
VITROS 5,1 F S/5600 (c)	6	6.11	8.3	8.1	134.8
Immulite 2000/2000 XPi	1	6.22	8.8	8.8	131.9
Immulite/Immulite 1000	2	8.69	11.4	5.1	147.1
Dimension Vista	2	9.62	11.4	11.9	157.5
Dimension XL/RXL/RXL MAX	14	7.95	13.9	6.1	112.6

lack of result harmonisation below current cut-points may be due to variable detection of some albumin forms by different assays. A second study using larger patient sample numbers and more methods may clarify this point.

Confirmatory Testing

Most guidelines recommend confirmatory (repeat) testing although repeat intervals and repeat sample types vary. PAWG draft recommendations are that initial positive ACR results should be confirmed by repeat measurement on 1-2 occasions within three months.

Key recommendations regarding pre-analytical and analytical aspects of urine albumin measurement from NKDEP/IFCC working group and/or PAWG are presented in Table 3.

Measurement of Total Protein in Urine

Measurement of total protein in urine has a number of limitations as a screening tool for proteinuria. Different methods may react disproportionately for each protein present in a given urine sample making standardisation impossible.[15] There is no universally accepted definition of proteinuria with normal varying from <150 to <300 mg/L and 23 to 100 mg/mmol creatinine. As well, urine total protein measurement is less robust at low levels of protein than is urine albumin measurement and it is insufficiently sensitive to detect all clinically important concentrations of urine albumin.[16] Some authors have even questioned the commonly held view that screening using urine albumin alone would miss tubular proteinuria.[17]

Methods for measuring urine total protein in Australasia are broadly divided between turbidimetry using benzethonium chloride (48%) and dye binding using pyrogallol red (46%) with only 18 of 265 laboratories using other methods. The end-of-cycle report for cycle 52 of the basic urine programme from RCPA QAP covering the period July to November 2010 shows that for a median value of 0.12 g/L, all participants reported values between 0.04 and 0.25 g/L (n = 266), while the interquartile range was significantly tighter at 0.11-0.15 g/L, and only one laboratory failed the allowable limit of +/- 0.1 g/L. Performance was less uniform at the higher median value of 2.07 g/L: all participants ranged from 1.02-2.66 g/L and 15 laboratories failed the allowable limit criteria of +/- 10%. However a majority performed well, with an interquartile range 1.97-2.14 g/L (Table 4).

Conclusion

Screening for proteinuria in chronic kidney disease should be done by measurement of urine albumin on a fresh, first morning void. A reference measurement system is being developed that will validate the current use of common decision limits and facilitate harmony of results between laboratories; this will be particularly important at low levels of urine albumin. In the meantime laboratories can improve patient outcomes by standardising sample collection and handling. PAWG has made recommendations to facilitate this in Australasian laboratories.

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Table 3. Key recommendations for urine albumin sample collection and handling derived from NKDEP/IFCC and or PAWG.

- The term 'urine albumin' should be used rather than 'micro-albumin'.
- Patients should be well at baseline. They should have no urinary tract infection, no acute febrile illness, no intense exercise within the previous 24 hours and not be menstruating.
- Recommended urine collection is a fresh, first morning void. A minimum of 5 mL should be collected.
- If a first morning void is not practicable, random spot samples are acceptable.
- Urine creatinine must also be measured.
- Samples not able to be delivered to the laboratory within 8 hours, should be refrigerated.
- Analysis should be performed on the day of receipt but samples can be stored for up to 7 days at 2-8 °C if necessary.
- Cloudy or particulate samples should be centrifuged prior to analysis.
- Positive ACR results must be confirmed, ideally on a fresh, first morning void, by repeat measurement on 1-2 occasions within 3 months.
- Prolonged storage should be at -70 °C; samples should not be stored at -20 °C.

Table 4. Instrument Summary Data. End of Cycle Report for Urine Total Protein. RCPA QAP Basic Urine Chemistry Programme, Cycle 52 July – November 2010. Statistics are derived from median performance over 12 samples (duplicates at six concentrations) for each instrument group as in Table 2. See the RCPA Chemical Pathology QAP website[18] for more detailed explanation of the statistical processes.

Instrument	No. Labs	SD	CV	Low 0.12	High 2.07
Roche Diagnostics Hitachi Cobas c701/c702	1	0.026	2.3	0.11	2.11
Roche Diagnostics Hitachi Cobas c501/c502	26	0.025	2.3	0.11	2.06
Dimension Xpand	6	0.025	2.4	0.10	1.94
INTEGRA 700/800	31	0.028	2.5	0.12	2.11
Dimension XL/RXL/RXL MAX	25	0.029	2.7	0.12	1.97
Roche Diagnostics Hitachi Modular	25	0.033	2.9	0.12	2.10
Roche Diagnostics Hitachi 912	3	0.036	3.1	0.17	2.11
Abbott ARCHITECT c4000/c16000	7	0.032	3.1	0.11	1.94
Abbott AEROSET	1	0.036	3.4	0.11	1.99
INTEGRA 400/400+	7	0.039	3.5	0.12	2.08
Beckman Coulter LX20/LX40	3	0.042	3.6	0.13	2.22
ADVIA1200	1	0.038	3.6	0.10	2.00
Beckman Coulter AU600/640	13	0.046	3.7	0.12	2.05
Beckman Coulter AU2700/AU5421/AU5432	12	0.046	3.7	0.15	2.24
Abbott ARCHITECT C8000	15	0.042	4.1	0.12	1.94
Roche Diagnostics Hitachi 917	2	0.049	4.3	0.15	2.10
ADVIA 2400	12	0.050	4.8	0.13	2.03
VITROS 5,1 F S/5600 (c)	7	0.036	4.9	0.10	1.73
Beckman Coulter UniCel DxC 600/601i	30	0.058	4.9	0.15	2.22
Dimension Vista	3	0.053	5.0	0.14	1.95
Beckman Coulter AU400	1	0.062	5.1	0.14	2.28
Beckman Coulter Synchron CX5	12	0.060	5.4	0.14	2.14
Spectrophotometric Plate Reader	1	0.078	6.0	0.13	2.47
VITROS 250/350	4	0.041	6.6	0.22	1.02
Beckman Coulter UniCel DxC 800	10	0.085	7.3	0.16	2.13
ADVIA 1650/1800	4	0.085	8.0	0.11	2.01
Vital Scientific SELECTRA E	1	0.123	10.2	0.16	2.17
ThermoFisher Scientific KoneLab 30i	1	0.273	26.2	0.25	1.84
Siemens Healthcare Diagnostics	1	0.556	41.2	0.04	2.66

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