ORIGINAL ARTICLE

Comparison of In-house Microalbumin Assay with Cobas C311 - Methodological Insights and Clinical Implications

Rukhsana Tumrani[1*], Afsheen Nigar[1], Mahnoor Chaudhry[1] and Maryum Ilyas[2]

[1]*Department of Pathology, Shalamar Medical and Dental College, Lahore, Pakistan*
[2]*Shalamar Hospital Laboratory, Lahore, Pakistan*

Abstract

Background: In clinical practice, urine albumin monitoring is a helpful technique for managing hypertension, diabetic nephropathy, and chronic kidney disease. Since urine albumin cannot currently be determined using standardized procedures or reference material, it is helpful to compare the various assays used in clinical laboratories.

Objective: To compare and verify the analytical performance of in-house microalbumin assay with COBAS c 311.

Material and Methods: After obtaining ethical approval from the institutional review board, a cross-sectional study was conducted in the Department of Research and Innovation, Shalamar Medical and Dental College, Lahore from September 2022 to December 2022. The leftover urine samples that fulfilled the inclusion criteria were collected from Shalamar Hospital Pathology Laboratory. The in-house microalbumin assay was prepared according to protocol. Urine samples with albumin levels less than 300 µg/ml estimated on Cobas c 311 were included. Urine samples of subjects, suffering from urinary tract infections were excluded. Comparative analysis between the two assays was performed by correlation, regression analysis, independent sample t-test and bland Altman plot.

Results: Of the total 150 non-diabetics (Group A), the mean age was 41±7.81 years while the mean age of diabetics (Group B) was 45±5.68 years. Linear regression and correlation studies with standards, patient samples and quality control samples showed reliable results. Linearity showed a strong correlation (r=0.9907), and average percentage recovery was optimal for calibrator-spiked (and water-spiked samples). The mean value estimated by in-house microalbumin assay in diabetics was 128mg/g and by Cobas C311 was 119mg/g with a mean difference of 9 was calculated. No statistically significant difference between the two methods was shown with a p-value of 0.109. In non-diabetics, the mean value estimated by in-house microalbumin assay was 11mg/g while by Cobas C 311 was 14mg/g with the difference between the two calculated as 3. No statistically significant difference between the two methods was found with a p-value of 0.087.

Conclusion: On the basis of this study, it is concluded that in-house microalbumin assay is simple, reliable and economical with a long shelf life and can be used as an initial screening test for the clinical management of patients with diabetic nephropathy and early detection helps in improvement in the management of these patients.

Keywords: *In-house microalbumin, comparison of method, methodological insights.*

INTRODUCTION

The most frequent cause of end-stage kidney disease is diabetic nephropathy. An essential marker of cardiovascular disease and hypertension, elevated urine albumin excretion is a hallmark of diabetic kidney impairment [1]. Frequent albuminuria screening leads to early identification and prompt treatment. According to international guidelines, all patients with type 2 diabetes and those with type 1 diabetes whose disease has lasted more than five years should have their albumin levels in their urine measured annually [1].

Urine albumin measurement is crucial for identifying and tracking renal disease. Because there is currently no reference system in place that consists of verified reference materials, as well as a reference measurement process, urine albumin measurement, is not standardized [1]. Diabetes, cardiovascular disease, and chronic kidney disease (CKD) can all be diagnosed and prognosed with urine albumin [2]. Urine collection methods and analytical measurement techniques must be taken into account by healthcare professionals when interpreting albumin values. The albumin concentration from a 24-hour urine collection has historically been used as the benchmark for calculating the amount of albumin expelled from the urine, or urine albumin excretion rate [2, 3].

Microalbuminuria occurs when the amount of albumin excreted in the urine is between 30 and 300 mg every 24 hours. Microalbuminuria is becoming more widely recognized as a sign of renal impairment. It has significant implications for the clinicopathological relationship with diabetic nephropathy and other cardiovascular problems in patients with or without diabetes, despite appearing to indicate passage of a relatively minimal quantity of protein excretion. In light of the patient's health perspective, early detection of microalbuminuria is thought to be the goal of health professionals to modify the treatment plan appropriately. Thus, it makes sense that knowledge of the precise pathophysiological importance of microalbuminuria

**Corresponding author: Rukhsana Tumrani, Department of Pathology, Shalamar Medical and Dental College, Lahore, Pakistan, Email: r.tumrani333@gmail.com*
Received: July 27, 2024; Revised: December 02, 2024; Accepted: December 04, 2024
DOI: https://doi.org/10.37184/jlnh.2959-1805.2.32

and its influence on prognosis in renal illnesses would be extremely beneficial in achieving improved therapeutic outcomes [4-6]. It is crucial to understand the variables that influence a patient's risk of developing microalbuminuria in type II diabetes [7, 8]. The study aims to compare and verify the analytical performance of the in-house microalbumin assay with Cobas c311. As there are no standardized assays available for the estimation of urine albumin, so comparing different assays would be helpful for the estimation of urinary albumin.

MATERIAL AND METHODS

After obtaining ethical approval from the institutional review board, a cross-sectional study was conducted in the Department of Research and Innovation, Shalamar Medical and Dental College, Lahore from September 2022 to December 2022. A batch of in-house reagents for microalbumin assay was prepared, which was enough for 1000 tests. Urine samples of non-diabetics n=150 (Group A) and of diabetics n=150 (Group B) were determined for albumin levels on Cobas c 311. A convenient sampling technique was applied to collect the left-over urine samples from the pathology laboratory. Urine samples with albumin levels less than 300 µg/ml estimated on Cobas c 311 were included. Urine samples of patients suffering from urinary tract infections were excluded. The in-house Elisa assay reagents were prepared using the chemicals available locally. The in-house assay components prepared were antibody-coated plates, enzyme conjugates, standards, quality control pools, substrate, stop solution, assay buffer and wash buffer. Cross-reaction studies were carried out by adding varying concentrations of BSA (Bovine serum Albumin) in assay buffer to see if there was any cross-reactivity with the primary antibody. Urine samples with Albumin concentrations in normal and high pathological range were diluted 20 and 40-fold using diluent buffer, to see if the diluted samples are comparable with undiluted. Recovery of human albumin was studied by adding 10 and 30 ug/ml of human albumin to 20 urine samples containing endogenous urinary albumin in the range of 2-8ug/ml.

Aliquots of 150 patients' 24-hour urine samples (remaining samples from standard medical care) were taken and kept in plastic tubes at -80°C. Five days was the longest period between collection and measurement. Before testing, samples were brought back to room temperature. The comparative test took six nonconsecutive days, or two weeks, to finish.

Data was analyzed using SPSS version 25. Comparison of a method performed by linear regression $y = ax + b$ (where y shows the result of the index method, a show the slope, x shows the value of the reference method and b shows the intercept), correlation studies, independent sample t-test and Bland Altman plot. P-value less than or equal to 0.05 was taken as statistically significant.

RESULTS

Of the total 150 non-diabetics (Group A), the mean age was 41±7.81 years while the mean age of diabetics (Group B) was 45±5.68 years. Linear regression and correlation studies with standards, patient samples and quality control samples showed reliable results. Linearity showed a strong correlation (r=0.9907), and average percentage recovery was optimal for calibrator-spiked (and water-spiked samples). Percentage recovery at base value 2.76ug/ml was 100.6% at spiking solution 10ug/ml and 99.6% at 30ug/ml spiking solution. While at base value 5.35ug/ml, percentage recovery was 100% at spiking solution 10ug/ml and 102.7% at 30ug/ml spiking solution. Percentage recovery at base value 7.73ug/ml was 100.1% at spiking solution 10ug/ml and 107.6% at 30ug/ml spiking solution.

Methodological Evaluation

Assay Parallelism

Three experimental pools studied for 2-fold and 4-fold dilutions and % recovery for these three pooled samples showed reliable results as shown in Table **1**.

Table 1: Results of three typical experimental pools studied for dilution, 2-fold and 4-fold dilutions.

Sample	Dilution	Observed value (µg/ml)	Expected value (µg/ml)	Recovery (%)
1	Undiluted	176	-	-
	2-fold	84	88	95.4
	4-fold	73	44	97.7
2	Undiluted	152.39	-	-
	2-fold	76.10	76.19	99.8
	4-fold	37.89	38.09	99.4
3	Undiluted	15.96	-	-
	2-fold	8.23	7.98	100.3
	4-fold	4.08	3.99	102.2

Linearity

Linearity showed a strong correlation r=0.9907 as shown in **Fig. (1)** average percentage recovery was optimal for calibrator-spiked (and water-spiked samples). Comparative analysis between the two assays was performed using correlation and regression analysis.

Recovery

Percentage recovery at base value 2.76ug/ml was 100.6% at spiking solution 10ug/ml and 99.6% at 30ug/

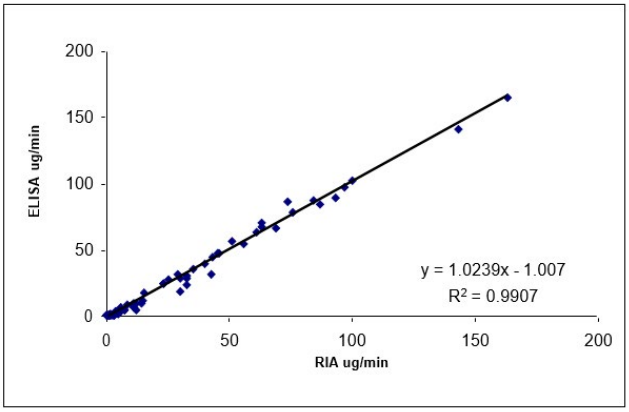

Fig. (1): Correlation studies of urine samples with in-house reagents and COBAS c 311.

ml spiking solution. While at base value 5.35ug/ml, percentage recovery was 100% at spiking solution 10ug/ml and 102.7% at 30ug/ml spiking solution. Percentage recovery at base value 7.73ug/ml was 100.1% at spiking solution 10ug/ml and 107.6% at 30ug/ml spiking solution (**Table 2**).

The mean value estimated by in-house microalbumin assay in diabetics was 128mg/g and by Cobas C311 was 119mg/g and the difference calculated was 9. No statistically significant difference between the two methods was shown with a p-value of 0.109. In non-diabetics, the mean value estimated by in-house microalbumin assay was 11mg/g while by Cobas C 311 was 14mg/g with the difference between the two calculated as 3. No statistically significant difference between the two methods was found with a p-value of 0.087 (**Table 3**).

T-test Statistics

Bland Altman has been shown in **Fig. (2)**. The Mean bias calculated between both methods was 0.15 with a standard deviation of 6.77. The upper limit of agreement was 13.69 and the lower limit of agreement was -13.39. The x-axis shows the average of both method's results and y axis shows the difference between both method's results. In this scatter plot, a difference of both method's measurements is plotted against the mean of two measurements. In this plot, it has been shown that more difference has been observed at higher values of microalbumin *i.e.* >150mg/g. Positive and negative bias is distributed equally on both sides at lower and higher values.

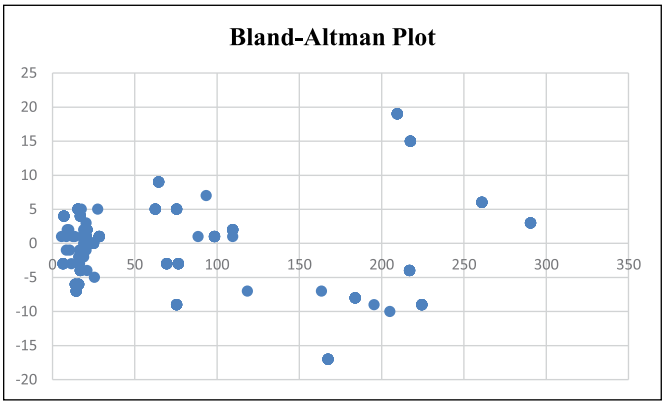

Fig. (2): Comparison between two methods by Bland Altmann plot.

DISCUSSION

A variety of methodologies are available to estimate the microalbumin level. On the basis of our study, it has been observed that in-house microalbumin assay shows comparable results with Cobas c311. The mean

Table 2: Results of three typical experimental pools studied for recovery estimation using 10 and 30µg/ml of albumin as spiking solution.

No.	Base value(µg/ml)	Spikingsol. (µg/ml)	Observedvalue (µg/ml)	Expected value (µg/ml)	Recovery (%)
1	2.76	10	12.84	12.76	100.6
		30	32.64	32.76	99.6
2	5.35	10	15.29	15.35	100
		30	35.93	35.35	102.7
3	7.73	10	17.74	17.73	100.1
		30	40.02	37.17	107.6

Table 3: Mean difference of results between in-house microalbumin assay and Cobas c311.

Population	Method	Mean Result (mg/g)	Difference	P-value
Diabetics	**In-house microalbumin assay**	128	9	0.109
	Cobas C311	119		
Non-Diabetics	**In-house microalbumin assay**	11	3	0.087
	Cobas C311	14		

value in diabetics and non-diabetics subjects estimated by both methods did not show a statistically significant difference between both methods with p-value>0.05 and linearity shows a strong positive correlation (r=0.997).

A study conducted by Molinario *et al.* showed a good linear relationship between the methods of urinary albumin and it can contribute to the standardization as well as harmonization of the assay used for estimation of urinary albumin [1]. We have found the comparable accuracy and precision of the two methods so the findings of the study conducted by Molinario *et al.* are consistent with our study.

A study conducted by Matte *et al.* found clinically significant differences between the two methods used for the estimation of urinary albumin. However, perfect agreement was found between two automated analyzers for albumin to creatinine ratio but there could be misinterpretation in progressive kidney disease patients. These findings are inconsistent with our study as we have found comparable accuracy and precision between the two methods [9].

Upon analysis at Atellica CH 930, four of the ACR values were stratified in lower ACR categories compared to Cobas 6000. Such classification discrepancies may result in an inaccurate assessment of the course of the patient's kidney illness. For instance, if the ACR is greater than 300 mg/g, a further clinical assessment of the kidney function (glomerular filtration rate, or GFR) is advised. However, it is important to note that the two platforms had flawless compliance in 37 of the 42 samples or 88% of the cases.

The study assesses how two widely used analytical platforms, which quantify both urine albumin and creatinine, affect ACR classification. However, a higher percentage of samples with urinary albumin concentrations (>1000 mg/L) would have been ideal as this would have increased the validity of the findings from the upper dilution range. The employment of various centrifugation programs in this investigation is another drawback. Before urine albumin analysis, centrifugation is advised [9-13].

It's unclear, though, if using various centrifuge programs has an impact on the albumin concentration readings. It is therefore possible that the outcomes of this investigation were influenced by the employment of various centrifugation programs prior to analysis. This worry would vanish if the samples were centrifuged using the same programs [9-13].

There is a need for well-defined reference material derived from human urine to harmonize urinary albumin assay [14].

In this study, the biological variation of the ACR was 11.8%, while the intra-individual biological variance of the AER on a 24-hour timed collection was 25.7%. Consequently, these data aided in defining analytical goals for albuminuria imprecision, with desired coefficients of variation (CV) for AER and ACR of 12.9% and 5.9%, respectively [15].

There are numerous approaches to identifying albuminuria.

However, due to the lack of a reference system that consists of verified reference materials and a reference measurement process, the quantification of urine albumin is not standardized [12, 13]. Therefore, efforts to standardize albuminuria measurement are currently being made by the National Institute of Standards and Technology (NIST), the National Kidney Disease Education Program (NKDEP), and the Working Group for the Standardization of Albumin Assays in Urine (WG-SAU) of the International Federation of Clinical Chemistry (IFCC) [16].

When read by a clinical operator (sensitivity 67%) rather than a laboratory professional (sensitivity 83%) as would be the case in real-world practice, a semiquantitative POC test (basically Clinitek, Siemens HealthCare Diagnosis, Tarrytown, USA) is not sensitive enough (76%) to rule out albuminuria in patients at risk for kidney disease according to a meta-analysis by McTaggart *et al*. It is therefore dubious why the results should be discussed with the patients right away, particularly if a laboratory test is required to confirm the outcome. However, the evidence-based necessary cut-off was satisfied by the quantitative point of care device (DCA, Siemens HealthCare Diagnostics, Tarrytown, USA) with a sensitivity of 96% and a sensitivity of greater than 95% [17].

When a clinical operator read the POC, however, sensitivity decreased to 91%. Although these techniques outperform traditional urine dipsticks for total urinary proteins, laboratory approaches should be used instead of POC testing for screening individuals with diabetes, chronic kidney disease, or those who are at risk for renal disease [18].

In 2014, Bachmann *et al*. conducted a comparison between 17 immunoassays (sixteen quantitative and one semiquantitative) and isotope dilution mass spectrometry. The primary cause of inconsistency among the standard kits was bias, with median biases ranging from -35% to 34% at 15 mg/L. Compared to the LC-MS/MS measurement, nine measurement techniques exhibited biases exceeding ±10% at the

clinically relevant threshold of 30 mg/L. Patients' risk of renal disease may be incorrectly classified as a result of the discrepancy in results [19].

Urine contains a variety of albumin forms, including partial degradation, glycated, and fragments. Albuminuria fragmentation could result from chemical changes made during sample preservation or from kidney breakdown [20].

LIMITATIONS

Reproducibility batch to batch and variation could not be checked due to a shortage of time.

CONCLUSION

On the basis of our study, it is concluded that in-house microalbumin assay is simple, reliable and economical with long shelf life and it can be used as an initial screening test for clinical management of patients with diabetic nephropathy and early detection helps in improvement in management of these patients.

ETHICAL APPROVAL

Ethical approval was obtained from the Institutional Review Committee of Shalamar Medical and Dental College, Lahore (REF letter No.: SSAHS-1RB/AL/60/2022 IRB no.: 0479). All procedures performed in studies involving human participants followed the ethical standards of the institutional and/ or national research committee and the Helsinki Declaration.

CONSENT FOR PUBLICATION

Written informed consent was taken from participants.

AVAILABILITY OF DATA

Data can be acquired from corresponding author upon request.

FUNDING

Declared none.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

ACKNOWLEDGEMENTS

Declared none.

AUTHORS' CONTRIBUTION

Dr. Rukhsana Tumrani: Data analysis, final approval of manuscript to be published

Dr. Afsheen Nigar: Manuscript writing, Literature review

Dr. Mahnoor Chaudhry: Data analysis, Discussion

Maryam Ilyas: Data collection

REFERENCES

1. Molinario R, Pocino K, Daloiso PD, Giannace A, Spirito G, Zuppi C. *et al*. Urinary albumin detection: comparison of two different methods. J Clin Lab Anal. 2016; 30(6), 888-91. DOI: https://doi.org/10.1002/jcla.21952 PMID: 27061674
2. Seegmiller JC, Miller WG, Bachmann LM. Moving toward standardization of urine albumin measurements. EJIFCC 2017; 28(4): 258-67. ECollection PMID: 29333145
3. Miller WG, Seegmiller JC, Lieske JC, Narva AS, Bachmann LM. Standardization of urine albumin measurements: status and performance goals. J Appl Lab Med 2017; 2(3): 423-29. DOI: https://doi.org/10.1373/jalm.2017.023614 PMID: 33636850
4. Waghmare P, Goswami K. Microalbuminuria: A Mere Marker or An Ominous Sign? J Assoc Physicians India 2016; 64(3): 61-5. PMID: 27731559
5. Fici F, Bakir EA, Beyaz S, Makel W, Robles NR. PAIT-survey-Prevalence of albuminuria in patients with diabetes and hypertension in Turkey. Prim Care Diabetes 2018; 12(6): 558-64. DOI: https://doi.org/10.1016/j.pcd.2018.08.008 PMID: 30327190
6. Tanaka H. Orthostatic proteinuria revisited: new clinical impact of the "old" clinical entity? Ann Transl Med 2020; 8(13): 814. DOI: https://doi.org/10.21037/atm.2020.02.181 PMID: 32793659
7. Chatzikyrkou C, Menne J, Izzo J, Viberti G, Rabelink T, Ruilope LM, *et al*. Predictors for the development of microalbuminuria and interaction with renal function. J Hypertens 2017; 35(12): 2501-9. DOI: https://doi.org/10.1097/hjh.0000000000001491 PMID: 29035939
8. Scurt FG, Menne J, Brandt S, Bernhardt A, Mertens PR, Haller H, *et al*. Systemic Inflammation Precedes Microalbuminuria in Diabetes. Kidney Int Rep 2019; 4(10): 1373-86. DOI: https://doi.org/10.1016/j.ekir.2019.06.005 PMID: 31701047
9. Mette B. Jensen, Ingunn Viken, Frederikke Høgh, Katja K. Jacobsen. Quantification of urinary albumin and -creatinine: A comparison study of two analytical methods and their impact on albumin to creatinine ratio. Clin Biochem 2022; 108: 5-9. DOI: https://doi.org/10.1016/j.clinbiochem.2022.06.014 PMID: 35787404
10. Bahar B, Tuncel AF, Holmes EW, Holmes DT. An interactive website for analytical method comparison and bias estimation. Clin Biochem 2017; 50(18): 1025-1029, DOI: https://doi.org/10.1016/j.clinbiochem.2017.08.008 PMID: 28823761
11. Westgard QC. Desirable Biological Variation Database specifications, Westgard.com. (2014). Available from: https://www.westgard.com/biodatabase1.htm
12. Birn H, Kamper AL, Ladefoged SA, Neland M, Randers E, Rehling M, *et al*. Analysemetoder og klinisk evaluering. Rekommandationer for vurdering af glomerulær filtrationsrate og albuminuri, Dansk Nefrologisk Selskab. (2015)

13. H. Martin. Laboratory measurement of urine albumin and urine total protein in screening for proteinuria in chronic kidney disease. Clin Biochem Rev 2011; 32(2): 97-102.
PMID: 21611083

14. Sindt C, Jørgensen HL. Statistiske metoder i biomedicin. BoD–Books on Demand; 2017.

15. Résimont G, Cavalier E, Radermecker RP, Delanaye P. Albuminuria in diabetic patients: how to measure it? A narrative review. J Lab Precis Med (JLPM) 2022; 7: 4.
DOI: https://doi.org/10.21037/jlpm-21-58

16. Seegmiller JC, Bachmann LM. Urine Albumin Measurements in Clinical Diagnostics. Clin Chem 2024; 70(2): 382-91.
DOI: https://doi.org/10.1093/clinchem/hvad174 PMID: 38321881

17. McTaggart MP, Newall RG, Hirst JA, Bankhead CR, Lamb EJ, Roberts NW, *et al*. Diagnostic accuracy of point-of-care tests for detecting albuminuria: a systematic review and meta-analysis. Ann Intern Med 2014; 160(8): 550-7.
DOI: https://doi.org/10.7326/m13-2331 PMID: 24733196

18. Résimont G, Piéroni L, Bigot-Corbel E, Cavalier E, Delanaye P. Urinary strips for protein assays: easy to do but difficult to interpret! J Nephrol 2021; 34(2): 411-32.
DOI: https://doi.org/10.1007/s40620-020-00735-y PMID: 32328900

19. Bachmann LM, Nilsson G, Bruns DE, McQueen MJ, Lieske JC, Zakowski JJ, *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): 471-80.
DOI: https://doi.org/10.1373/clinchem.2013.210302 PMID: 24281781

20. Benz-de Bretagne I, Roger C, Carlier MC, groupe de travail SFBC, SFNDT, SNP. Urinary albumin measurement methods. Ann Biol Clin (Paris) 2018; 76(6): 633-7.
DOI: https://doi.org/10.1684/abc.2018.1390 PMID: 30543187