Journal of Clinical Laboratory Analysis 23 : 314–318 (2009)

An Improved Microalbumin Method (μALB_2) with Extended Analytical Measurement Range Evaluated on the ADVIA® Chemistry Systems

Pradip Datta[1] and Amitava Dasgupta[2]*

[1]*Siemens Healthcare Diagnostics, Tarrytown, New York*

[2]*Department of Pathology and Laboratory Medicine, University of Texas Medical School, Houston, Texas*

Quantitative determination of albumin (ALB) in human urine is important to assess kidney functions in a variety of diseases. Recently, Siemens released an improved Microalbumin assay (μALB_2) to measure urinary ALB on the automated, random access ADVIA 1650/1800, ADVIA 2400, and ADVIA 1200 Chemistry Systems. We evaluated analytical performances of this new method. All ADVIA Chemistry Systems use the same microalbumin reagent packs, μALB_2 calibrators, and commercial controls. The within-run and total CVs of the improved method with two-level BioRad Liquichek Urine Chemistry controls (~2 and 9 mg/dl ALB) and a urine pool (~29 mg/dl ALB) on all ADVIA Chemistry systems were <4.1 and $<6.1\%$, respectively (40 replicates per sample). The analytical range/linearity of the method (all ADVIA systems) was from 0.3 mg/dl to the ALB concentration in the highest level of calibrator (~38–42 mg/dl). The improved method (μALB_2) on the ADVIA 1650/1800 (y) correlated well with both the Beckman DXC 800 Microalbumin and the old microalbumin method on the ADVIA 1650/1800 analyzers. The improved method showed $<10\%$ interference with 16 chemicals from acetaminophen to uric acid that may be present in urine. The improved method has a minimum of 60 days' on-system stability on all systems with the calibration frequencies of (with/without a Reagent Container insert) 20/30 days (ADVIA1200), 50/60 days (ADVIA1650/1800), and 20/60 days (ADVIA2400). No prozone was observed with the method on any platform up to the highest ALB concentration tested in a sample (4,000 mg/dl). J. Clin. Lab. Anal. 23:314–318, 2009.

Key words: microalbumin; ADVIA analyzer; Beckman analyzer; interference

INTRODUCTION

Albumin (ALB) is the major plasma protein with a molecular weight of 67,000 Da that is responsible for much of the osmotic force of the blood. Other than an oncotic agent, ALB is also the major binding protein of many drugs. ALB acts as an extracellular scavenger, which leads to oxidation of ALB molecules. This process reduces function of the ALB and eventually leads to its degradation. In disease, oxidation of ALB is increased, leading to rapid aging of ALB and eventually its degradation (1).

In a healthy person, only a small amount of ALB (up to 30 mg/day) is excreted in 24 hr urine. An increased urinary ALB excretion is an established test for early detection of renal disease and is also recognized as a risk factor for cardiovascular disease. In addition, data indicate that random urinary ALB to creatinine ratio correlate well with 24 hr urinary ALB excretion measurement (2). The presence of small amount of ALB (microalbumin) in the urine of patients with type-2 diabetes is probably the most important early sign of the onset of vasculopathy, and associated target organ damage including the brain, heart, and the kidneys. Microalbuminuria also identifies patients at higher risk of developing cardiovascular disease especially more

*Correspondence to: Amitava Dasgupta, Department of Pathology and Laboratory Medicine, University of Texas Medical School, 6431 Fannin, MSB 2.292 Houston, TX 77030.
E-mail: Amitava.Dasgupta@uth.tmc.edu

Received 21 April 2009; Accepted 4 June 2009
DOI 10.1002/jcla.20341
Published online in Wiley InterScience (www.interscience.wiley.com).

strict control of blood pressure, preferably below 130/80, as well as tight glycemic and lipid control (3). Yesim et al. reported that although diabetes and hypertension may cause microalbuminuria, it was not detected in obese women without diabetes or hypertension (4). Microalbuminuria and proteinuria are also believed to be precursors of sickle cell nephropathy (5). In addition, elevated urinary ALB may also be associated with hypertension, some lipid abnormalities, several immune disorders, as well as other conditions such as vigorous exercise, blood in the urine, urinary tract infection, dehydration, and treatment with certain medications. Waldron et al. reported that patients with exercise induced myocardial ischemia have pre-exercise microalbumin excretion and is not associated with exercise (6). Patients with HIV infection may show abnormal urinary protein excretion including microalbumin (7).

Siemens Healthcare Diagnostics has recently released the Microalbumin_2 Method (μALB_2), with an extended analytical range, for the determination of microalbumin in human urine samples using the automated random-access ADVIA Chemistry analyzers. We evaluated the analytical performance of this new method and would like to report our findings.

MATERIALS AND METHODS

The ADVIA Chemistry Microalbumin_2 (μALB_2) method (Siemens Healthcare Diagnostics, Deerfield, IL) uses polyethylene glycol (PEG) enhanced immunoturbidimetry to measure the ALB concentration in urine samples. In this method, as run on the ADVIA 1650/1800, ADVIA 2400, or ADVIA 1200 systems, sample is reacted with the reagents R1 that contains 6% PEG and incubated at 37°C for 5 min. Subsequently, reagent R2 containing goat antibodies to human ALB is added. After another 5 min, the turbidity in the reaction matrix is measured at 340 nm (end-point). By constructing a standard curve from the absorbances of six-level standards, at ALB concentrations of about 0, 1, 4, 10, 20, and 40 mg/dl, the ALB concentration in a sample can be determined. All ADVIA Chemistry μALB_2 methods use the same calibrators: blank (water) and the five-level ADVIA Chemistry μALB_2 calibrators. Of the ADVIA Chemistry systems used in our studies, the ADVIA 1650/1800 and 2400 systems use a 5× sample pre-dilution. The dilution is done automatically by the system using the system diluent (saline). From the diluted sample multiple assays can be run. Furthermore, for samples containing ALB concentration greater than 38 mg/dl, the system can dilute the prediluted samples by additional tenfold, reruns the assay, and reports the result after multiplying the observed concentrations by the dilution factor. The ADVIA 1200 system, on the other hand, uses undiluted neat sample. And, it can also trigger auto-rerun (tenfold dilution) for samples containing ALB concentration greater than 38 mg/dl.

All ADVIA Chemistry systems use the same reagent packs, calibrator, and controls. The same calibrator values and control ranges are used across all three platforms. The method has a minimum of 60 days on-system stability on all systems. The calibration is stable for 20 days on the ADVIA 1200 and 2400 and 60 days on the ADVIA 1650/1800 without any Reagent Container Insert. With the Reagent Container Insert, calibration holds up to 30 days on the ADVIA 1200) and 60 days on the ADVIA 1650/1800 and 2400.

The Beckman Microalbumin method was run on the Beckman DXC 800 system using manufacturer recommended materials and protocol. This assay is also a turbidimetric assay. In this assay urinary ALB combines with anti-ALB antibody forming an insoluble complex compound and the signal is measured at 380 nm. The anti-ALB antibody is also goat antibody specific to human ALB.

RESULTS

Precision, Linearity, and Detection Limit

We evaluated the precision of the ADVIA Chemistry μALB_2 method on the ADVIA 1650/1800, 2400, and 1200 systems by assaying the two-level BioRad Liquichek assayed Urine Chemistry Controls with ALB concentrations of approximately 2 and 9 mg/dl and two human urine pools at clinically relevant urine ALB concentrations of approximately 3 and 30 mg/dl for 10 days, with two runs/day and two replicates/run (a total of 40 replicates/sample). Imprecision estimates, computed according to CLSI document EP5-A2, ''Evaluation of Precision Performance of Quantitative Measurement Methods—Approved Guideline, Second Edition' and we observed excellent precision for this new assay (Table 1). In general total CVs were all significantly below 5%. The worst precision of total CV of 6.1% was observed with ADVIA 1650/1800

TABLE 1. Precision of the New μALB_2 Method on Various ADVIA Analyzers

	ADVIA 1650/1800	ADVIA 2400	ADVIA 1200
Within	4.1% @ 1.9 mg/dl	2.1% @ 1.7 mg/dl	3.8% @ 2.4 mg/dl
	2.1% @ 2.9 mg/dl	1.6% @ 2.8 mg/dl	2.2% @ 2.9 mg/dl
	1.8% @ 9.1 mg/dl	0.9% @ 8.9 mg/dl	1.3% @ 10.4 mg/dl
	1.2% @ 29.3 mg/dl	0.8% @ 28.8 mg/dl	1.4% @ 29.8 mg/dl
Total	6.1% @ 1.9 mg/dl	3.8% @ 1.7 mg/dl	4.7% @ 2.4 mg/dl
	3.3% @ 2.9 mg/dl	2.9% @ 2.8 mg/dl	3.7% @ 2.9 mg/dl
	2.5% @ 9.1 mg/dl	1.1% @ 8.9 mg/dl	1.8% @ 10.4 mg/dl
	1.7% @ 29.3 mg/dl	1.0% @ 28.8 mg/dl	1.8% @ 29.8 mg/dl

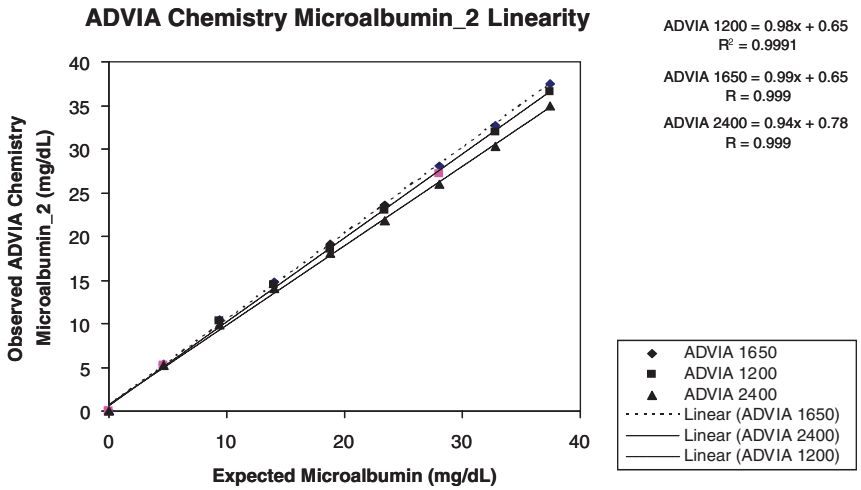

Fig. 1. Linearity of ADVIA 1200, 1650, 2400 analyzer for μALB_2.

TABLE 2. Establishment of Detection Limit of the Assay

	Absorption (mean+2 SD)		
ADVIA system	System 1	System 2	MDC[a] (mg/dl)
1650/1800	0.0032	0.0106	0.08
2400	0.0013	0.0014	0.07
1200	0.0047	0.0076	0.29

[a]MDC: Minimum detected concentration.

analyzer for a control Bio-Rad specimen containing only 1.9 mg/dl of ALB.

The linearity and analytical range of the ADVIA Chemistry μALB_2 method on the ADVIA 1650/1800, 2400, and 1200 systems were assessed by assaying nine-level dilution of a high urine pool with saline (system diluent) on the ADVIA systems. We observed good correlation between target microalbumin concentrations and observed microalbumin concentrations thus establishing excellent linearity of this new method (Fig. 1).

The minimum detectable concentration (detection limit) of the new method on three ADVIA Chemistry systems were determined from the μALB_2 concentrations equivalent to the Absorption (mean+2 × standard deviation) for the zero calibrator (water) using a total of 40 replicates over 10 days. The detection limit was established as 0.3 mg/dl based on the highest value observed among all three analyzers (Table 2).

The ADVIA Chemistry systems allow flagging and auto-rerun, with tenfold increased dilution, for samples with ALB levels higher than the upper assay range (~38 mg/dl), thus extending the reportable assay upper range to 380 mg/dl. The accuracy and precision of the auto-rerun feature were assessed with two samples containing 18 and 40 mg/dl ALB. They were first assayed without rerun, and then were with auto-rerun after tenfold dilution automatically done on the system (both in five replicates, on all three systems). The imprecision and mean of both sets of results were comparable.

Method Comparison

We compared the ADVIA 1650/1800 μALB_2 method with two commercial microalbumin assays: the Beckman DXC 800® Microalbumin and the current ADVIA 1650/1800 μALB (old assay), by assaying 50 and 98 urine samples, respectively. Of the 50 samples used in the first method comparison study (range: 0.8–114.6 mg/dl), 10 samples had ALB concentration higher than the assay range (>38 mg/dl) and their results were obtained by the auto-rerun feature of the ADVIA Chemistry systems (as described above). The new method showed excellent correlation with the Beckman assay. Using the x-axis as the values obtained by the Beckman assay (reference method) and the y-axis as the value obtained by the new method using ADVIA 1650/1800 analyzer, we observed the following regression analysis (Fig. 2):

$$y = 0.97x + 0/36 \quad (r = 0.998).$$

As expected using other ADVIA analyzer, this new method also showed excellent correlation with the Beckman microalbumin assay (data not shown).

We also observed excellent correlation between the old ADVIA microalbumin (μALB, x-axis) method and the new microalbumin method (μALB_2, y-axis) produces the following regression analysis (Fig 3):

$$y = 0.99x - 0.07 \quad (r = 0.99).$$

As expected the new method performed well between various ADVIA analyzers. For example when the performance of this assay on ADVIA 1650/1800 was

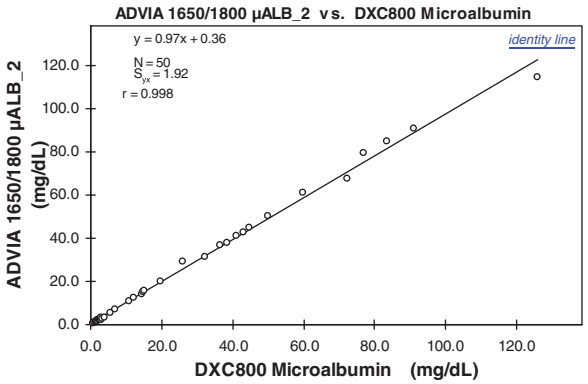

Fig. 2. Linear regression equation showing excellent correlation between this new microalbumin method with an existing Beckman microalbumin assay.

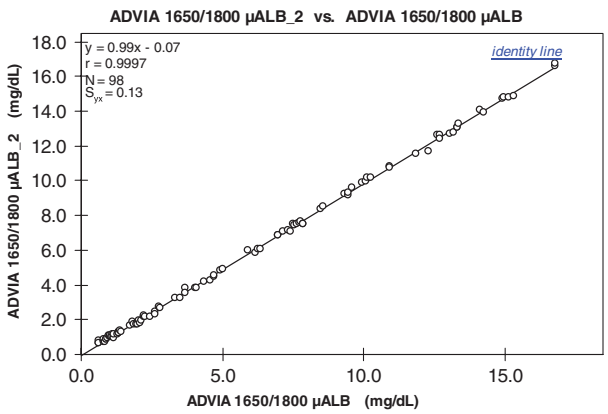

Fig. 3. Correlation between the old microalbumin method with the new microalbumin method using ADVIA 1650 analyzer.

compared with the ADVIA 2400 analyzer, we observed the following regression equation:

$$y = 0.97x - 0.00 \quad (0.99).$$

Interference Study and Prozone Effect

Interference for the new method on all three platforms was evaluated by spiking a human urine pool at µALB_2 with approximately 3 mg/dl with the following 16 chemicals; acetaminophen, ascorbic acid, calcium, citrate, creatinine, glucose, hemoglobin, hippuric acid, potassium chloride, magnesium, sodium chloride, oxalate, phosphorus, salicylate, urea, and uric acid. Multiple levels of interfering substances were tested. We observed no significant interference (recovery of $< \pm 10\%$ from the baseline control sample) even in the presence of these interferences at concentrations many folds higher than supraphysiological concentrations (Table 3).

To determine the Prozone limit (''hook'') of the method, we spiked human urine pool samples with varying concentrations of human ALB (up to 4,000 mg/dl), and ran them in ADVIA Chemistry µALB_2 method. As shown in Table 4, the absorbances for such samples were always above the absorbance observed for the highest calibrator, and no hook was observed up to the highest ALB concentration tested on any ADVIA Chemistry system.

DISCUSSION

Microalbumin is measured in both random urine specimens and 24 hr urine specimen using various assays

TABLE 3. Data Showing No Significant Interference of 13 Analytes in the New Microalbumin Assay

		% Recovery (lowest to highest)[a]	
Analyte tested	Range of concentration	ADVIA1650	ADVIA2400
Acetaminophen	12.5–100 mg/dl	93.4–96.2	89.5–99.2
Ascorbic acid	62.5–500 mg/dl	93.2–98.3	94.3–97.9
Calcium	50–400 mg/dl	96.7–98	90.3–95.4
Citrate	62.5–500 mg/dl	98.3–101.7	97.2–101.3
Creatinine	62.5–500 mg/dl	94.5–101.7	97.4–106.9
Glucose	625–5000 mg/dl	92.0–96.3	91.6–94.8
Hemoglobin	62.5–500 mg/dl	100.0–104.8	99.4–101.7
Hippuric acid	50–400 mg/dl	91.4–96.6	93.9–97.9
Oxalate	3.75–30 mg/dl	95.2–101.9	96.5–100.2
Phosphorus	50–400 mg/dl	95.0–100.0	93.2–97.9
Salicylate	31.25–250 mg/dl	95.3–100.2	93.2–98.2
Urea	50–400 mg/dl	94.3–99.2	93.3–104.8
Uric acid	12.5–100 mg/dl	96.7–98.4	99.7–100.8

Results obtained using ADVIA 1200 analyzer is not shown but are very similar to the data presented.
[a]Interference study was performed using a control microalbumin specimen containing 3.0 mg/dl of microalbumin.

TABLE 4. Data Showing No Prozone or Hook Effect with Samples Containing Albumin Up to 4,000 mg/dl: with Signal Ratio (with Respect to the Signal of the Highest Calibrator) > 1.00, the Samples Read > 40 mg/dl (Upper Analytical Range)

		Absorbance Ratio: Sample/Highest Calibrator		
Sample	Albumin (mg/dl)	ADVIA1650/1800	ADVIA2400	ADVIA1200
Highest calibrator	40	1.00	1.00	1.00
Sample-1A	500	3.46	3.40	3.22
Sample-1B	1,000	3.28	3.25	2.95
Sample-1C	2,000	3.19	3.07	2.70
Sample-1D	4,000	2.96	3.00	2.50

that can be automated on a chemistry analyzer. Assadi et al. reported that measurement of microalbumin to creatinine ratio in a second-voided urine specimen is a simple and realizable method for monitoring microalbuminuria in diabetic patients and may replace the need of quantitative determination of microalbumin in 24 hr urine specimen (8). As technology progresses, older radioimmunoassay for determination of urinary microalbumin are mostly replaced by nonradioactive-based immunoassay. More recently, point of care devices are also available for determination of microalbumin in urine specimens. In one study, the authors concluded that Clintek-Microalbumin screening test can be used to exclude microalbuminuria and the method is reliable. However, for values over 3 mg/dl, the presence of ALB in urine must be confirmed by the timed overnight collection of urine (9). Ng et al. developed tow in-house ELISA assay for the determination of microalbumin concentrations in urine specimens (10).

The new method we evaluated has good specificity, sensitivity, linearity and is free from common interferences. In addition, this assay has wider analytical measurement range compared with the previous version, ADVIA Chemistry microalbumin. With a wider analytical range (the auto-rerun option available on the ADVIA Chemistry systems can extend the range further as needed), the new method can detect urine ALB in both low (~3 mg/dl) as well as high (~30 mg/dl) concentrations in the same assay. We conclude that this new microalbumin method is useful for application on any ADVIA platform for determination of routine microalbumin concentrations in urine specimens in clinical laboratories.

REFERENCES

1. Soeters PB. Rationale for albumin infusion. Curr Opin Clin Nutr Metab Care 2009 March 30;12:258–264.
2. Guy M, Newall R, Borzomato J, Karla PA, Prive C. Diagnostic accuracy of the urinary albumin: creatinine ratio determined by the CLINITEK Microalbumin and DCA 2000+ for the rule out of albuminuria in chronic kidney disease. Clin Chim Acta 2009; 399:54–58.
3. Weir MR. Microalbuminuria in type 2 diabetes: an important overlooked cardiovascular risk factor. J Clin Hypertens (Greenwich) 2004;6:134–141.
4. Yesim TE, Ugurlu S, Caglar E, et al. Investigation of microalbuminuria in nondiabetic normotensive obese women. Intern Med 2007;46:1963–1965.
5. McKie KT, Hanevold CD, Hernandez C, et al. Prevalence, prevention and treatment of microalbuminuria and proteinuria in children with sickle cell disease. J Pediatr Hematol Oncol 2007;29:140–144.
6. Waldon JS, Baoku Y, Hartland AJ, et al. Urine microalbumin excretion in relation to exercise-induced electrocardiographic myocardial infarction. Med Sci Monit 2002;8:CR 725–727.
7. Kimmel PL, Umana WO, Bosch JP. Abnormal urinary protein excretion in HIV-infected patients. Clin Nephrol 1993;39:17–21.
8. Assadi FK. Quantitation of microalbuminuria using random urine samples. Pediatr Nephrol 2002;17:107–110.
9. Meinhardt U, Ammann RA, Fluck C, et al. Microalbuminuria in diabetes mellitus: Efficacy of a new screening method in comparison with timed overnight urine collection. J Diabetes Complications 2003;17:254–257.
10. Ng L, Teng LC, Ng ML, et al. Microalbuminuria measurements by two in house ELISA methods. Malays J Pathol 2000;22:73–78.