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Comparison of Instrument-Read Dipsticks for Albumin and Creatinine in Urine With Visual Results and Quantitative Methods

Michael J. Pugia,[1] John A. Lott,[2]* Karl E. Luke,[1] Zak K. Shihabi,[3] Frank H. Wians, Jr.,[4] and Lawrence Phillips[5]

[1]*Diagnostics Business Group, Bayer Corporation, Elkhart, Indiana*
[2]*Department of Pathology, The Ohio State University, Columbus, Ohio*
[3]*Department of Pathology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, North Carolina*
[4]*Department of Pathology, University of Texas Southwestern Medical School, Dallas, Texas*
[5]*Emory Clinic, Atlanta, Georgia*

Three hospital sites evaluated the Bayer two-pad urine dipstick as a screening test for microalbuminuria. One pad estimates albumin concentrations between 10 and 150 mg/L, and the second estimates creatinine values between 300 and 3,000 mg/L. The Boehringer Mannheim (BMD) Micral® dipstick was also compared and evaluated. The accuracy of the dipsticks was judged by comparison with cuvet-based immunonephelometry for albumin and to standard rate-Jaffé methods for creatinine; these assays were well standardized and controlled and were assumed to give accurate values. Precision of these methods and that of the dipsticks was determined by multiple assays of control materials. Visual or instrument (Clinitek 50® or 100®) evaluation of the Bayer or visual checks of the BMD albumin dipstick pad with patients' urines gave clinically acceptable accuracy. The albumin/creatinine ratio from the Bayer dipsticks gave better accuracy for albumin excretion than the albumin pads alone from either manufacturer. This ratio should permit making a good estimate of the 24-hr albumin excretion in a randomly collected urine. J. Clin. Lab. Anal. 12:280–284, 1998.

Key words: microalbuminuria; albumin concentrations; creatinine values; albumin/creatinine ratio

INTRODUCTION

Microalbuminuria, defined here as albumin excretion >20 mg/24 h, is a harbinger of renal disease and a risk factor for cardiovascular events such as myocardial infarction or stroke (1); this is particularly true in patients with non-insulin dependent diabetes (2,3). It also predicts the development of clinically important proteinuria and early mortality in maturity-onset diabetes (4). In one study, a finding of an albumin excretion of >200 mg/24 hr in hypertensive men was a predictor of mortality and cardiovascular morbidity. This was not so if the albumin excretion was ≤ 30 mg/L (5). Patients with essential hypertension, especially African-Americans, often show microalbuminuria (6,7). Redon, et al.(8), found a significant correlation between the albumin concentrations in urine and the severity of hypertension.

Ordinary dipsticks used to estimate urine albumin excretion have inadequate sensitivity to identify patients with microalbuminuria; immunoassays or high-sensitivity dye-binding methods must be used. Based on earlier work (9), we propose a hierarchy of the accuracy of methods for estimating albumin in urine from the least to the most accurate:

1. A dipstick that measures albumin in a random urine reliably down to about 10 mg/L;
2. The same as #1 but with a correctly collected 24-hr urine;
3. A dipstick test that measures both albumin and creatinine in urine so an albumin/creatinine ratio and an estimate of the 24-hr albumin excretion can be calculated;
4. Reliable quantitative cuvet chemistry measurements of albumin and creatinine on a random urine and calculation of the 24-hr albumin excretion;
5. The same as #4 but with a correctly collected 24-hr urine.

*Correspondence to: John A. Lott, Ph.D., Department of Pathology, The Ohio State University Medical Center, Starling Loving M-368, Columbus, OH 43210. E-mail: lott.1@osu.edu

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The measurement of albumin alone on a random urine is not recommended, because it is dependent on the patient's fluid intake and state of hydration. A highly dilute urine could give an albumin of <20 mg/L in a patient with true microalbuminuria; conversely, a concentrated urine could lead to a false-positive result. An accurate 24-hr collection is ideal but is a commonly unmet challenge. If the urine creatinine is also measured, the ratio of albumin to creatinine eliminates the factors of volume and collection time in the expression,

$$[(\text{mg albumin})/(\text{L}\bullet\text{min})]/[(\text{g creatinine})/(\text{L}\bullet\text{min})] = \text{mg albumin/g creatinine.}$$

Ginsberg, et al. (10) and Shaw, et al. (11), showed that the above ratio is highly correlated with the 24-hr protein excretion. Others made the same finding and proposed that a ratio of <100 mg albumin per g of creatinine is normal (12). This ratio is better if a correction is made for the body surface area, A, in m^2, by multiplying the above by (A/1.73). Estimates of body surface area are available as nomograms that are based on the height and weight of the individual (13). For example, an adult weighing 80 kg and with a height of 147 cm has a surface area of 1.73 m^2. Other nomograms must be used for children.

The normal 24-hr creatinine excretion shows between-person variation, and a better estimate of the 24-hr albumin excretion is simply:

$$(\text{g creatinine/24 hr})/(\text{mg albumin/g creatinine}) = \text{mg albumin/24 hr.}$$

The first term is measured or calculated from the Cockroft and Gault equation (14), and the second term is obtained from the analysis of albumin and creatinine. The above equation is valid for estimating the 24-hr excretion of both albumin or protein. The word, "estimate" should be stressed, because the diurnal variation of albumin excretion is largely unknown, and the calculated 24-hr creatinine excretion is an estimate with at least a ± 10%–15% uncertainty. Normal persons and some patients with hypertension show a decrease in blood pressure and albumin excretion when recumbent, i.e., their albumin excretion varies with posture (15).

Quantitative, cuvet-based methods for albumin and creatinine are highly desirable for measuring albumin, but for screening purposes, the cost is difficult to justify. A dipstick that gives an estimate of both albumin and creatinine in urine should give clinically useful results at low cost. However, quantitative methods are needed if small changes in the 24-hr albumin excretion must be detected; here, dipsticks will not have sufficient accuracy.

The principal goal of this study was to evaluate a two-pad dipstick that tests for albumin and creatinine to see if it is suitable as a screening device for microalbuminuria. We wanted to compare the dipstick results with accepted quantitative methods to learn if the dipsticks were sufficiently accurate for this purpose. Another questions was if visual evaluation of the dipsticks is satisfactory or if instrument reading of the light reflectance from the pads is superior.

MATERIAL AND METHODS

Specimens From Patients

At each of the three evaluation sites, about 140 freshly collected urines were obtained for a total of about 420 specimens; they were either single void or 24-hr specimens from normal ambulatory or hospitalized patients with a variety of diagnoses. The evaluation sites are given in the Acknowledgments. About 90% of the specimens were not refrigerated or frozen and were tested within 2 hr of collection. The rest were either refrigerated and tested within seven days or frozen at –20°C and tested within 30 days.

Preparation of Contrived Urines

Fresh urine from normal adult volunteers was pooled, and any protein present was removed by ultrafiltration using a 10,000 dalton cutoff filter from Amicon, Inc. (Beverly, MA). Two urine pools were prepared: one had 300 mg/L creatinine and 20 mg/L albumin; the other contained 2,000 mg/L creatinine and 100 mg/L albumin. They were assayed in duplicate with all methods as described below at each of the three sites over a period of 10 days. We used Pentex® human serum albumin (Bayer, Kankakee, IL) to establish the desired albumin concentrations in the contrived urines, and reagent grade creatinine to supplement the urines to the values above. The Pentex albumin was 98% pure as determined by electrophoresis on cellulose acetate followed by densitometry. The concentration of albumin was traceable to the Reference Preparation for Proteins in Human Serum (RPHS), a certified reference material with an albumin value developed conjointly by the College of American Pathologists (as CAP Reference Preparation for Serum Proteins) and the Bureau Communitaire de Reference (as CRM 470: BCG designation). The concentration of creatinine was traceable to the National Institute of Science and Technology (NIST) Standard Reference Material (SRM 14) creatinine (16).

Aliquots of the contrived urines were stored at –20°C, and the specimens were thawed at room temperature for at least 4 hr before use. The creatinine values were determined on a Roche Fara II® analyzer (Roche Diagnostic Systems, Sommerville, NJ) with their reagents and procedure.

Assay Methods

Bayer dipsticks

At each site, the urines were assayed with the dipsticks by visual observation using two readers, by the dipsticks and

TABLE 1a. Precision of the Quantitative Albumin and Creatinine Methods at the Three Sites

Test[a]	Location (n)[b]	Mean	Percent CV	Test	Location (n)[b]	Mean	Percent CV
Albumin	All sites (60)	107.1	6.57	Creatinine	All sites (60)	29.9	6.85
	Site 1 (20)	105.4	6.38		Site 1 (20)	30.5	6.52
	Site 2 (20)	106.5	6.02		Site 2 (20)	29.0	5.61
	Site 3 (20)	109.3	7.33		Site 3 (20)	30.3	7.62
Albumin	All sites (60)	21.6	10.5	Creatinine	All sites (60)	191.1	2.69
	Site 1 (20)	22.6	9.87		Site 1 (20)	193.5	1.96
	Site 2 (20)	21.2	5.96		Site 2 (20)	188.8	3.30
	Site 3 (20)	21.1	13.8		Site 3 (20)	191.2	2.32

[a]All concentrations in mg/L.
[b]Number of determinations. Duplicate assays were performed for albumin and creatinine at each of the three sites for 10 days.

TABLE 1b. Precision of Instrument-Read Clinitek Albumin and Creatinine Dipsticks Albumin, mg/L (n = 60)[a]

Expected value	Found value	Percent CV	Instrument-read by
21.6	21.9	10.2	Clinitek 50
21.6	21.4	7.6	Clinitek 100
107.1	99.4	8.1	Clinitek 50
107.1	103.6	9.2	Clinitek 100
	Creatinine, mg/L (n=60)[a]		
Expected value	Found value	Percent CV	Instrument-read by
299	287	2.6	Clinitek 50
299	300	2.6	Clinitek 100
1909	1970	3.3	Clinitek 50
1909	1910	4.0	Clinitek 100

[a]Readings from two instruments at each of three sites for 10 runs. The same lot of strips was used at each site.

instrument reading of the reflectance, and by the quantitative methods for albumin and creatinine as described below. The readers were found to not be color blind based on a standard test (17).

The Bayer two-pad dipstick measures both albumin and creatinine in urine. A visual estimate of the albumin concentrations is possible using a color comparison chart with nominal albumin values of 10, 30, 80, and 150 mg/L. Each specimen was also tested with C-Stix® (Bayer) for ascorbic acid and with Multistix 10SG® (Bayer) for blood and protein to eliminate those with hematuria and/or frank proteinuria. The microalbumin dipstick has an upper useable limit of ca. 150 mg/L. Concentrations of albumin at and above 150 mg/L give the maximum color on the albumin pad. Specimens with >25 mg hemoglobin per liter give a falsely increased albumin value (9).

The creatinine pad is read the same way and compared to its color chart; the nominal concentrations are 300, 1,000, 2,000, and 3,000 mg/L. The Bayer dipstick can also be instrument-read with either a Clinitek® 50 or Clinitek® 100 reflectance photometer.

Boehringer dipstick

All urines were also tested with the Micral-Test® strips (Boehringer Mannheim Corporation, Indianapolis, IN) and visual evaluation according to the manufacturer's instructions (18,19). The Micral-Test® is a gold sol immunometric dipstick method. The color comparison chart gives nominal albumin values of "negative," 20, 50, and 100 mg/L.

Quantitative albumin assays

Two immunonephelometric methods for albumin were used. The first, the micro-albumin reagent on the Array® analyzer from Beckman (Beckman Instruments Inc., Fullerton, CA) was used at two sites according to the manufacturer's instructions. The second method utilized the SPQII reagent (Incstar Corp., Stillwater, MN) on a the Roche COBAS-FARA® II analyzer according to the manufacturer's directions, and was used at the third site.

There was no statistical difference ($P > 0.05$) between the results for the controls for albumin or creatinine at the three sites allowing us to combine the albumin and creatinine data. The precision of the tests is given in Table 1a.

Quantitative creatinine assays

Each of the three evaluators used a different instrument for the creatinine assay: the Bayer Technicon RA 1000 (Bayer Corp., Tarrytown, NY), the Dade Paramax (Dade Corp., Miami, FL), and the Dade DuPont XL analyzer, each with the manufacturer's reagents and rate-Jaffé procedure.

RESULTS AND DISCUSSION

Reproducibility of Instrument-Read Dipsticks on Contrived Urines

The two contrived urine pools were analyzed in duplicate by dipstick at each of the three sites for 10 days giving 60 results for both albumin and creatinine. The combined data for the three sites are given in Table 1b. The Clinitek 50 and 100 have a built-in calibrating mechanism that measures the reflectance from a standard solid surface; other calibration is not needed. The expected mean values were calculated from the albumin added to the urine pools; the mean creatinine was determined from the sum of the endogenous plus the added creatinine.

TABLE 2. Accuracy of Albumin Dipsticks

Pad value on Bayer color chart	Quantitative value	Albumin-uria	Visual reading with Bayer dip-sticks	Instrument-read with Clinitek 50	Instrument-read with Clinitek 100	Visual reading with Micral dipsticks	Pad value of Micral color chart
10[a]	≤20	Absent[b]	88.8% (326/367)[c]; 0 too low[e], 41 too high[e]	88.7% (386/435); 0 too low, 49 too high	85.5% (372/435); 0 too low, 63 too high	85.3% (303/355); 0 too low, 52 too high	Negative[d]
30	20 to ≤55	Borderline	76.6% (98/128); 20 too low, 10 too high	61.3% (106/173); 39 too low, 28 too high	56.1% (97/173); 38 too low, 38 too high	54.4% (80/147); 11 too low, 56 too high	20
80	>55 to 115	Present	58.2% (39/67); 26 too low, 2 too high	63.1% (65/103); 30 too low, 8 too high	55.3% (57/103); 30 too low, 16 too high	77.2% (61/79); 4 too low, 14 too high	50
150	>115	Present	80.2% (65/81); 16 too low, 0 too high	72.4% (76/105); 29 too low, 0 too high	77.1% (81/105); 24 too low, 0 too high	57.3% (51/89); 38 too low, 0 too high	100

[a]Albumin values in mg/L.
[b]"Absent" was based on quantitative result of ≤20 mg/24 h. We considered quantitative values of 20 to 30 mg/L as being "borderline" and above that as "Present."
[c]For example, 326 of 367 urines gave a pad color of "10" when read visually; the 367 urines gave a quantitative result of ≤20 mg/L, and we assumed that these values were correct. The difference, 41 urines ("too high"), gave a pad reading greater than "10."
[d]Note that the color chart values of the Micral dipsticks are different than that of the Bayer dipsticks.
[e]Of all the readings stated as "too low" or "too high," 99.3% were within one color block of the expected value.

Accuracy of the Albumin Dipsticks in the Assay of Patients' Urines

Table 2 shows the accuracy of the albumin dipsticks when read visually using a color chart or by reflectance photometry using the Clinitek 50 or 100 according to the manufacturer's instructions. In all cases, the quantitative methods were assumed to give the correct value for albumin and creatinine in urine. For example, of 367 urines that contained < 20 mg albumin per liter by the quantitative assay, 326 gave a visual reading of "10." Forty-one specimens gave dipstick values of " > 10 mg/L," i.e., the dipstick overestimated the albumin by one concentration block and read as "30 mg/L" on the dipsticks in all cases. At higher concentrations of albumin, the visually determined values tended to underestimate the albumin concentration. The same was true for the strips read by the Clinitek 50, Clinitek 100, and the visually evaluated Micral strips. But in 99.3% of all cases shown in Table 2 when the dipstick reading was either "too low" or "too high," it was off by only one color block. Surprisingly, visual reading was more accurate than the instrument reading on the Clinitek instruments in the "30 mg/L" range. The specificity of the dipsticks at < 20 mg albumin per liter was good, and there were only 10%–15% false positives.

Table 3 gives the quantitative values of the albumin/creatinine ratios in mg albumin/g creatinine plus the same information for the visually and instrument-read dipsticks. The pads of course do not give the albumin/creatinine ratios; these must be calculated from the observed albumin and creatinine values. The ratios of albumin to creatinine as determined on the Clinitek 50 or 100 for the 30–300 mg/L range showed about a 10% better accuracy than the single albumin pad assayed on the same instruments (see Table 3). Thus there is a distinct advantage in measuring the albumin/creatinine ratios rather than the albumin alone.

TABLE 3. Accuracy of Albumin/Creatinine Ratios[a]

Ratio of pad values on chart	Ratio of quantitative values	Albuminuria[b]	Visually determined ratios	Clinitek 50 determined ratios	Clinitek 100 determined ratios
<30	<30	Absent	89.1% (352/395)[c]; 0 too low, 43 too high	89.2% (422/473); 0 too low, 51 too high	88.8% (420/473); 0 too low, 53 too high
≥30 to 300	≥30 to 300	Present	61.6% (188/305); 53 too low, 64 too high	71.5% (193/270); 68 too low, 9 too high	70.3% (189/269); 71 too low, 9 too high
>300	>300	Present	92.3% (12/13); 1 too low, 0 too high	66.3% (69/104); 35 too low, 0 too high	67.6% (69/102); 33 too low, 0 too high

[a]All values in mg albumin/g creatinine. The color pad reading for albumin was divided by the color pad reading for creatinine to give the values shown here.
[b]Based on the quantitative value being <30 mg albumin/g creatinine.
[c]For example, 352 urines gave pad color ratios of <30 when read visually, and the quantitative method gave 395 results with the ratio being <30; 43 urines gave dipstick ratios greater than this.

TABLE 4. 24-h Creatinine Excretion According to the Cockcroft and Gault Equation (14)

Age (years)	Men 60 kg	Women 60 kg	Men 70 kg	Women 70 kg	Men 80 kg	Women 80 kg	Men 90 kg	Women 90 kg
20	1.44[a]	1.22	1.68	1.43	1.92	1.63	2.16	1.84
30	1.32	1.12	1.54	1.31	1.76	1.50	1.98	1.68
40	1.20	1.02	1.40	1.19	1.60	1.36	1.80	1.53
50	1.08	0.92	1.26	1.07	1.44	1.22	1.62	1.38
60	0.96	0.82	1.12	0.95	1.28	1.09	1.44	1.22
70	0.84	0.71	0.98	0.83	1.12	0.95	1.26	1.07

[a]Calculated creatinine excretion per 24 h.

Creatinine Excretion

The 24-hr creatinine excretion is quite constant in normal individuals; however, it varies with age, weight, and gender (10). Table 4 shows some representative examples of normal 24-h creatinine excretion as calculated from the Cockcroft and Gault equation (14).

CONCLUSIONS

Both the Bayer and Micral dipsticks for albuminuria are easy to use, inexpensive, and give acceptable accuracy as screening methods. In all cases here, we assumed that the quantitative cuvet chemistries performed by us for albumin and creatinine gave the correct value. Because the Bayer strips also give the creatinine concentration, the albumin/creatinine ratio can be determined, and an estimate of the urinary albumin loss within 24 hours can be made. In a future study, we plan to test the hypothesis that there is a further gain in accuracy of the albumin loss if the calculated creatinine excretion is corrected for the age, weight, and gender of the patient. We found that the albumin/creatinine ratio from the dipsticks gave a better estimate of the urinary loss of albumin as compared to the albumin value alone. The dipsticks are useful for screening; when abnormal values are obtained, they should be confirmed by quantitative methods. The convenience and ease of use favors the screening for albuminuria with dipsticks since the added creatinine pad permits the testing of random urine specimens and a clinically useful estimate of the 24-hr albumin excretion can be obtained from these data.

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