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Multisite Evaluation of a New Dipstick for Albumin, Protein, and Creatinine

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The goal of our study was to perform a multisite evaluation of a new urine dipstick called Multistix PRO™ (Bayer, Elkhart, IN), which has reagent pads for the simultaneous assay of urinary albumin, protein, and creatinine. Patients' urine specimens were assayed at four sites with these dipsticks and with the familiar Bayer Multistix® 10SG dipsticks for protein. The new dipstick pads for albumin are impregnated with bis (3′,3″-diiodo-4′,4″-dihydroxy-5′,5″-dinitrophenyl)-3,4,5,6-tetrabromo-sulfonephthalein (DIDNTB) dye. These dipsticks also have a novel pad that estimates urinary creatinine using the peroxidase activity of the copper-creatinine complex. We determined the interlaboratory agreement of these dipsticks by comparing dipstick results to values obtained by quantitative analytical methods. We found that dividing the dipsticks' albumin or protein results by the creatinine concentration reduced the number of false-positive albumin or protein values observed in concentrated urines, and reduced the number of false negatives in dilute urines. The ratio of albumin to creatinine, or protein to creatinine gives a better measure of albumin or protein excretion. Compared to reading by eye, the dipstick results agreed better with the quantitative assays when they were read by a reflectometer (Bayer Clinitek). J. Clin. Lab. Anal. 15:231–235, 2001.

Key words: albuminuria; dye-binding assays; proteinuria; reflectance photometry; test evaluation

INTRODUCTION

It is now well established that even small increases in urinary protein or albumin excretion are early predictors of kidney failure and end-stage renal disease (1). If the patient also has diabetes and/or hypertension, the risk of developing kidney disease is even greater (2,3). Proteinuria is also a harbinger of mortality in patients with diabetes following coronary artery bypass grafting (4). Identification of patients who are at increased risk for kidney or related disorders is clearly worthwhile, and simple back-office testing is now available for the reliable detection of albuminuria and/or proteinuria. The goals of our study were to determine the accuracy of the dipstick assays for albumin and protein when compared to quantitative methods, to determine intersite variability of dipstick results, and to judge if dividing the albumin and/or protein results by the specimen's creatinine concentration reduces the number of false-positive or false-negative dipstick results for albumin and protein.

MATERIAL AND METHODS

Quantitative Assays

In our study, we assumed that the quantitative, cuvet-based results were accurate and could be used in judging the accuracy of the dipstick results for albumin, protein, and creatinine. Albumin in controls and in patients' urines were assayed at all sites using immunonephelometry. Two sites used a Beckman (Fullerton, CA) Array, one site used a Roche (Nutley, NJ) COBAS Integra, and one used a Dade (Miami, FL) Paramax. In all cases, the instrument makers' reagents and instructions were followed without modifications. For total protein, all sites used the Sigma pyrogallol red method ("Microprotein-PR8" method, No. 611-A; St. Louis, MO) for total urinary protein in a Bayer Opera analyzer, Beckman CX3,

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Roche Integra, or Roche Mira instrument. For creatinine, all the evaluators used a rate-Jaffé procedure on one of the above instruments.

Quality Control Specimens

We used three unique controls; all contained 10 g/L NaCl, 10 g/L urea, and 25 g/L of KH_2PO_4, plus varying concentrations of albumin, protein, and creatinine, as shown in Table 1A. All three controls were assayed by the quantitative methods at all sites and on each of 12 days to obtain between-day estimates of precision. We also obtained between-day quality control data for the two kinds of dipsticks, as described below (Table 1B). A judgment of the between-site agreement was based on the results for the controls as obtained by the quantitative methods.

Dipsticks

Bayer Multistix PRO™ dipsticks (hereafter termed "PRO") were used according to the manufacturer's instructions. The dipsticks include reagent pads using bis (3′,3″-diiodo-4′,4″-dihydroxy-5′,5″-dinitrophenyl)-3,4,5,6-tetrabromo sulfonephthalein (DIDNTB) dye for the detection of albumin at ≥80 mg/L (5). Also present on the dipsticks was a pad for protein that is based on tetrabromophenol blue (TBPB) for detection of protein at ≥300 mg/L, and a pad for creatinine that uses the peroxidase activity of a copper-creatinine complex (6). With these dipsticks, it is possible to determine the albumin/creatinine and protein/creatinine ratios. A negative protein result on a urine that has a creatinine of ≤100 mg/L indicates that the specimen is too dilute to obtain a reliable albumin/creatinine or protein/creatinine result. We also used the currently available Multistix® 10SG dipsticks. These are designated here as "TRACE" because they detect "trace" or higher concentrations of protein.

Assigned Ranges for Dipstick

We used artifactual urine pools with known concentrations of albumin, protein, and creatinine to test the color responses of the dipsticks. The dipsticks' responses can be adjusted during manufacture by changing the dye concentration, buffer, and other ingredients used to impregnate the dipstick pads. For example, the albumin dipstick pads were manufactured to give a positive dipstick response for albumin just above 80 mg/L, confirmed with quantitative assays, and to give a positive response for protein just above 300 mg/L, also confirmed with quantitative assays.

Specimens From Patients

Two hundred random or first morning urine specimens were collected at each of four sites (Ohio State University Medical Center, Bowman Gray School of Medicine, South West Washington Medical Center, and Fairview University Medical Center) from inpatients and outpatients with various diagnoses. The specimens were tested within 12 hr of collection or frozen on the day of collection, thawed overnight at 4°C, and tested the following day. All specimens were assayed in duplicate with the quantitative methods at each site. Dipstick results were obtained in duplicate by the visual observation

TABLE 1A. Combined quality control data for the quantitative assays at the four sites[a]

	Albumin, mg/L			Protein, mg/L			Creatinine, mg/L		
Control no.	Expected result	Found mean	% CV	Expected result	Found mean	% CV	Expected result	Found mean	% CV
1	NA[b]	NA	NA	NA	NA	NA	500	496	5.8
2	250	223	3.3	250	262	7.3	NA	NA	NA
3	50	48	3.0	50	37	26.0	2000	1907	2.0

[a]We found no statistical difference ($P > 0.05$) for the quality control results from the four sites. There are 96 data points for each control: 4 sites × 2/day × 12 days.
[b]NA means that this analyte was not added to the artifactual urine. We prepared artifactual urine pools with varying concentrations of albumin, protein, or creatinine, shown above as "expected result."

TABLE 1B. Combined quality control data for dipstick assays at the four sites[a]

	Albumin			Protein			Creatinine		
Control no.	Expected result, mg/L	Exact agreement, percent of specimens	±1 color block, percent of specimens[b]	Expected result, mg/L	Exact agreement, percent of specimens	±1 color block, percent of specimens	Expected result, mg/L	Exact agreement, percent of specimens	±1 color block, percent of specimens
1	NA	NA	NA	NA	NA	NA	≥500	84	100
2	150	100	100	100	95	100	NA	NA	NA
3	Negative	100	100	Negative	100	100	≥2000	57	100

[a]Dipsticks were analyzed on a Clinitek 50 reflectometer. NA means this analyte was not added to the artifactual urines.
[b]The dipstick results were within one color pad (above or below) the expected color pad.

of two readers and by Clinitek® model 50, 100, 200+, or 500 reflectance analyzers. All sites evaluated the strips visually and by the Clinitek 50 and 100 reflectometers. Three sites also tested all of their specimens on the Clinitek 200+ and 500 analyzers. We rejected data from a few specimens that had a negative protein result and a creatinine of ≤100 mg/L. In most cases, we suspected water contamination of the specimens.

RESULTS AND DISCUSSION

Quality Control Data for Quantitative Methods

The reproducibility of the quantitative methods for the analyses of the three artifactual urines is shown in Table 1A. The CVs ranged from 2% to about 26%, showing that the quantitative methods had adequate precision. The dilute specimens with protein concentrations of ≤50 mg/L had a CV of 26%. We compared the quality control data from the four sites for albumin, protein, and creatinine to determine if merging the data was acceptable. Statistical analysis showed that the results on the controls from the four sites were not statistically different ($P > 0.05$ in all cases), and that merging the data for the controls and patients was acceptable.

Statistical Method

We used the Mann-Whitney nonparametric test in all trials to estimate statistically significant agreement or lack thereof.

Reproducibility of Instrument-Read Dipsticks

The three control solutions were analyzed in duplicate by the PRO dipsticks at each of the four sites for 12 days, giving 96 between-day results for albumin, protein, and creatinine, and the ratios of albumin to creatinine, and protein to creatinine. The combined data for the four sites are given in Table 1B. Table 2 shows the agreement of the results for patients' urine with the quantitative ("correct") results and those obtained with the PRO or TRACE dipsticks. Dipsticks were read visually and by reflectance photometry with one of the four models of the Clinitek analyzers. Each site used two of the four different Clinitek analyzers and analyzed 200 patients' urine with their instruments. For example, in Table 2, in the first row of data from the TRACE dipstick results, the positive predictive value (PPV) was lowest for visual reading (76%), better with the Clinitek 50 (83%), and highest with the Clinitek 500 (87%) ($P < 0.01$, visual vs. any of the machine methods). From the rest of the data, left to right, it is apparent that reading the dipsticks with a reflectance photometer gives a better PPV and better negative predictive value (NPV) in nearly every case. Visual reading with the PRO dipsticks gave PPVs close to that obtained with the reflectometers, which is an advantage. Our data suggest that reflectometer reading and use of the PRO dipsticks gives the best agreement with the quantitative methods.

Accuracy of Dipsticks on Patients' Specimens

The albumin/creatinine and protein/creatinine ratios from the 800 patients were obtained with the quantitative methods and the PRO dipsticks. The urines were also tested with the TRACE dipsticks for protein only. As usual, we considered the quantitative method results to be correct. Values of 80 mg albumin/L, 300 mg protein/L, 80 mg albumin/g creatinine, or 300 mg protein/g creatinine were considered to be the upper limits of the reference ranges with the quantitative methods. The false-positive results for the dipsticks are shown in Fig. 1a. A false positive was defined as a result of > trace by the

TABLE 2. Agreement table, visual and reflectometers reading for patients' urines[a]

Agreement of *visual* reading for albumin or protein with quantitative methods.[b] All values in percent			Agreement of *machine* reading for albumin or protein with quantitative methods.[b] All values in percent.			
Dipstick	Statistical test	Visual reading	Clinitek 50	Clinitek 100	Clinitek 200+	Clinitek 500
TRACE dipstick	PPV[c]	76	83	84	85	87
	NPV[d]	95	98	97	96	95
PRO dipstick	PPV[c]	93	92	87	91	95
	NPV[d]	91	97	98	93	95
Agreement of *visual* reading for albumin/creatinine or protein/creatinine with quantitative methods. All values in percent			Agreement of *machine* reading of protein/creatinine ratios or albumin/creatinine ratios with quantitative methods. All values in percent.			
Protein/creatinine	PPV[c]	75	81	83	78	79
	NPV[d]	73	84	83	86	85
Albumin/creatinine	PPV[c]	92	92	89	94	94
	NPV[d]	81	91	94	98	98

[a]Both compared to Quantitative Methods.
[b]A specimen was considered as abnormal if either the quantitative albumin was >80 mg/L (abnormal) or the quantitative protein was >300 mg/L (abnormal). The quantitative methods were assumed to give the correct value.
[c]Positive predictive value (PPV) = (number of confirmed positives by quantitative assay/total number of positives by dipstick assay) × 100.
[d]Negative predictive value (NPV) = (number of confirmed negatives by quantitative assay/total number of negatives by dipstick assay) × 100. All dipstick results were compared to the quantitative assay values. We assayed 800 urines frm patients; 200 specimens came from each site.

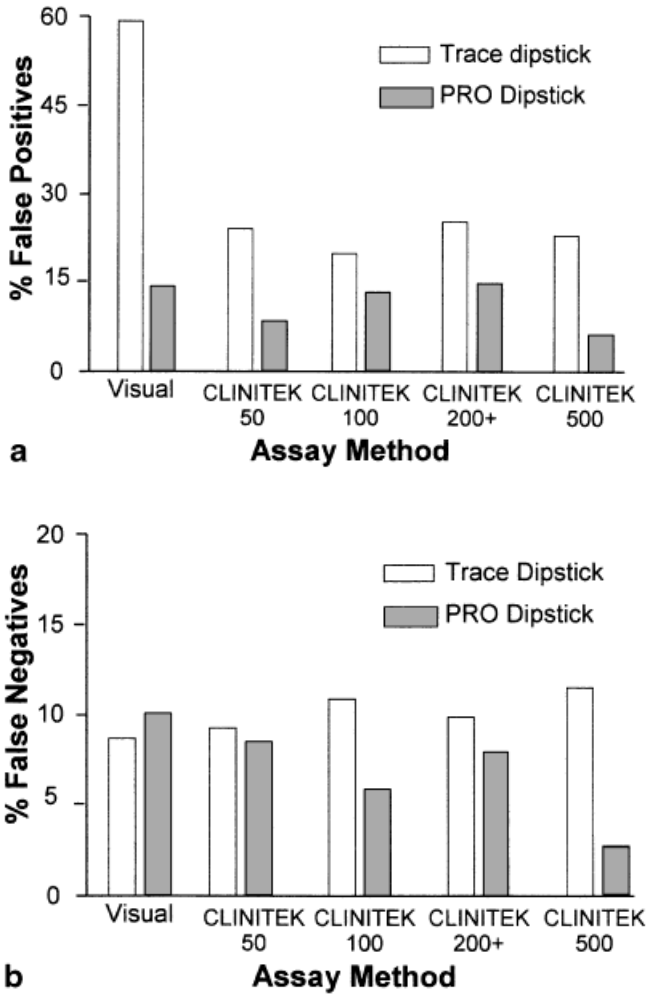

Fig. 1. **a:** False-positive rate for the TRACE (protein only) and the PRO dipsticks (albumin/creatinine) for the 800 patients. The dipstick results were compared to the quantitative methods that were assumed to be accurate. All tests were performed in duplicate and the values were averaged. The PRO dipsticks' albumin results divided by their creatinine concentrations gave fewer false positives in all five assay methods when compared to the TRACE dipstick results. **b:** False-negative rate of the same group of 800 patients shown in part a. Use of the reflectometers and dividing the PRO values by their creatinines consistently gave fewer false-negative results.

TRACE dipsticks or an albumin/creatinine ratio >80 mg with the PRO dipsticks when these tests were within the reference limits by the quantitative methods.

As shown in Fig. 1a, the false-positive rate with the PRO dipsticks (the albumin/creatinine value) was lower in comparison to the TRACE dipsticks (protein only) for all five assay methods. Without exception, there was a statistically significant difference ($P < 0.05$) between the PRO dipstick results (albumin/creatinine) and the TRACE dipstick results (protein only). Visual reading of the TRACE dipsticks gave the greatest percentage of false-positive results.

The false-negative rates are shown in Fig. 1b. The false-negative rate with the PRO dipsticks (albumin/creatinine) was lower in comparison to the TRACE (protein only) dipsticks in four of the five dipstick assay methods (Fig. 1b). Dividing albumin or protein results by a specimen's creatinine makes sense, given an earlier finding (7) of a reduced number of false positives and false negatives by this technique. There was no statistically significant difference ($P > 0.05$) between the visual reading of the PRO and TRACE dipsticks. We found fewer false-negative values ($P < 0.01$) by the Clinitek 100 and 500 reflectometers when each was compared to visual reading.

Effect of Urine Concentration on Dipstick Results

We grouped the 800 patients' data according to their creatinine concentrations. Dilute urine may give a false-negative

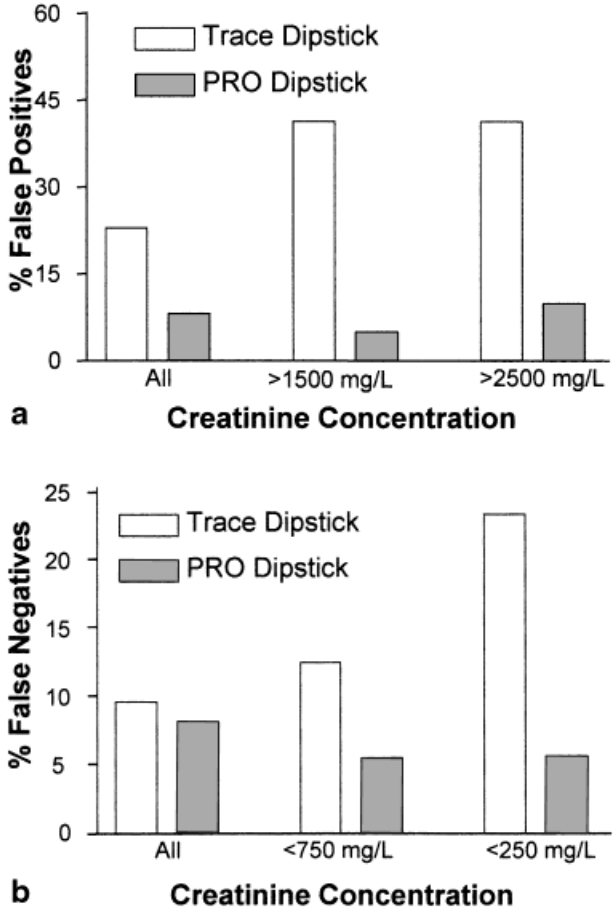

Fig. 2. a: False-positive rate of specimens with high creatinine concentrations (last pair of bars). Dividing the PRO results by the specimens' creatinine value greatly reduced the number of false-positive results. All dipsticks were read with a Clinitek 50. The data include results for all 800 urines ("All"), 168 urines with a creatinine of >1,500 mg/L, and 47 urines with >2,500 mg/L. **b:** False-negative rate of specimens with low creatinine concentrations (last two sets of bars). Dividing the PRO results by the specimens' creatinine value greatly reduced the percentage of false-negative results. The data include results for all 800 urines ("All"), 315 urines with creatinines of <750 mg/L, and 52 urines with <250 mg/L. All dipsticks were read with a Clinitek 50.

result; the albumin or protein may be abnormal, but in a dilute urine the albumin or protein concentrations may be below the cutoff. Also, we showed earlier that concentrated urines tend to give false-positive results for both albumin and protein (7).

We show in Fig. 2a that the PRO results divided by the specimens' creatinine showed fewer false positives than the TRACE results without a creatinine correction. The differences were statistically significant ($P < 0.01$) for each of the three pairs. Patients' specimens with low concentrations of creatinine are shown in Fig. 2b. Urines with creatinine concentrations of <250 mg/L by the quantitative method showed many false-negative results with the TRACE dipsticks. With dilute urines and assay by the PRO dipsticks (albumin/creatinine), there were fewer false negatives. We also found a significant difference in results for specimens with creatinines <750 mg/L for the PRO vs. TRACE dipsticks ($P < 0.001$).

CONCLUSIONS

We have strong evidence that the readings of dipstick pads with a reflectometer gives more accurate values than visual readings. We also found that correcting albumin or protein values by dividing these values by their creatinine concentrations led to a marked decrease in the number of false-positive and false-negative results. Nephrologists have known for a long time that a single dipstick test for albumin or protein is often unreliable in patients with highly dilute or concentrated urines. Dividing these values by the specimens' creatinine concentration improves the reliability of the testing (7). The PRO dipstick tests are easy to perform and are suitable for point-of-care testing.

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