Comparison of Six Commercial Techniques in the Measurement of Microalbuminuria in Diabetic Patients

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OBJECTIVE — To evaluate the advantages and drawbacks of six commercially available techniques in the measurement of microalbuminuria in diabetic patients.

RESEARCH DESIGN AND METHODS — Timed overnight urine samples from 75 patients in our diabetic clinic were tested with 2 qualitative tests (Micral-Test and Microbumintest) and assayed with 4 quantitative tests, which used different methodologies: double-antibody RIA, RID, IT, and NEPH. All tests were commercially available. The double-antibody RIA was taken as the golden standard. All urine samples were either negative or trace by Albustix (Ames, Elkhart, IN). Interobserver variation for the 2 qualitative tests was assessed by asking 12 experienced nurses to read the color changes on the dipsticks and the tablets simultaneously, using test solutions with albumin concentrations of 16, 32, and 64 mg/L.

RESULTS — The 75 urine samples contained 0–154.2 mg/L of albumin. Correlation coefficients of RID, NEPH, and IT with RIA were 0.970, 0.975, and 0.974, respectively. Intra-assay and interassay CVs ranged from 1.36–11.5%. Microbumintest had a higher sensitivity (100 vs. 75%), but lower specificity (82.5 vs. 87.3%) than Micral-Test. Considerable interobserver variation existed in the matching of colors for both tests. Discrepancies were especially significant with Microbumintest, with 8 of 12 nurses misreading the 32 mg/L level.

CONCLUSIONS — Correlations of the 3 quantitative methods with RIA were all >0.970. Choice of test may depend more on considerations such as time, space, number of specimens to be handled, and availability of instruments. Both qualitative tests showed a relatively low specificity. Positive tests must be confirmed with quantitative assays before microalbuminuria is diagnosed. Microbumintest had a higher sensitivity, but its unacceptably high interobserver variation and lower specificity were serious drawbacks.

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RIA, RADIOIMMUNOASSAY; RID, RADIAL IMMUNODIFFUSION; NIDDM, NON-INSULIN–DEPENDENT DIABETES MELLITUS; IDDM, INSULIN-DEPENDENT DIABETES MELLITUS; CV, COEFFICIENT OF VARIATION; IT, IMMUNOTURBIDIMETRY; NEPH, NEPHELOMETRY; AER, ALBUMIN EXCRETION RATE.

Because microalbuminuria was found to predict future development of overt diabetic nephropathy (1–3), screening of diabetic patients for an increased AER has become part of routine diabetic care. Such screening involves large numbers of patients; therefore, a simple, inexpensive, and sensitive test is necessary. Depending on the number of patients each handle and the financial and technical resources available. Different clinics may need to adopt different tests for practical use.

In this study, we compared the performance of 2 qualitative and 3 quantitative tests in the measurement of microalbuminuria with RIA to determine their relative advantages and drawbacks. All assays were commercially available.

RESEARCH DESIGN AND METHODS

Urine samples

Timed overnight urine samples (75) were collected from 75 patients in our diabetic clinic. Twenty-four patients were men, 51 were women; ages ranged from 23–81 yr, with a mean of 57.1 yr. Twenty-seven were IDDM patients, and 48 were NIDDM patients. The duration of diabetes ranged from 7 to 30 yr, with a mean of 13.8 yr. All urine samples were either negative or trace by Albustix. The samples were stored as 2-ml aliquots at −30°C with sodium azide added. Assays were performed within 4 wk of sample collection. The samples were thawed only once, before assaying, and were not centrifuged. Dilutions were performed only when necessary for the particular sample.

Assay methods

RIA. RIA was performed with a double-antibody technique (Albumin RIA kit, Pharmacia, Piscataway, NJ). Albumin in the sample competed with a fixed amount of ^{125}I-labeled albumin for the binding sites of the specific antibodies. Bound and free albumin were separated

Table 1—*Results of RIA, RID, IT, and NEPH in the measurement of 75 urine samples for microalbumin*

	RIA	RID	IT	NEPH
ALBUMIN CONCENTRATION RANGE (MG/L)	0–129.5	0–97.8	0–154.2	0–119.7
MEAN ALBUMIN CONCENTRATION (MG/L)	10.4 ± 17.5	8.4 ± 16.2	12.6 ± 18.1	8.9 ± 17.4
AER (MG/MIN)	0–210	0–180	0–204	0–244
SAMPLES WITH AER > 20 MG/MIN(%)	12 (16.0)	11 (14.6)	16 (21.3)	10 (13.3)

Data for AER are means ± .

by addition of a second antibody immunoadsorbent, followed by centrifugation and decanting. The radioactivity in the pellet was measured with a γ-counter. Albumin concentration in the sample was inversely proportional to the radioactivity (4).

RID. RID was performed with a single RID technique (5). Wells in an agarose gel layer containing monospecific antiserum to human albumin were filled with samples. The diameters of the precipitates were read with an electronic RID-plate reader (Binding Site Ltd., Marburg, Germany); VLC-Partigen albumin (Behring, La Jolla, CA) concentrations in the samples could be read from a curve plotted from the standards.

IT. This method depended on the formation of a turbid solution when albumin in the sample reacted with a specific antibody. The turbidity was measured with a spectrophotometer (Albusure QNT, Cambridge Life Sciences, Cambridge, U.K.) in the absorbance mode. The absorbance was proportional to the albumin concentration in the sample (6,7).

NEPH. This test required a nephelometer (Beckman, Brea, CA) and microalbumin kit. Albumin in the sample formed light-scattering centers when it reacted with a specific antibody to form antigen-antibody complexes. Based on the scatter signal, the calibrated nephelometer gave results in terms of albumin concentrations. Antigen-excess testing was built into the system (8).

Immunometric dipstick. The Micral-Test (Boehringer Mannheim, Mannheim, Germany) is a qualitative test based on an immunochemical principle. Albumin in the sample was bound by a soluble conjugate of antibodies and marker enzyme β-galactosidase. Unbound conjugates were removed by passage via a separate zone containing immobilized human albumin so that only the conjugate-albumin complexes would reach the reaction zone. Here, the enzyme β-galactosidase reacts with a substrate to produce a red dye; after exactly 5 min of the dye the intensity is directly related to the concentration of albumin in the urine sample. A measurement of ≥20 mg/L was considered positive. The test was performed by dipping the dipstick into urine for 5 s and reading the color change produced after 5 min.

Microbumintest. This qualitative test (Ames, Miles, Elkhart, IN) like Albustix, was based on the protein-error-of-indicators principle: At a constant buffered pH, the intensity of color is dependent on the concentration of the albumin present in the sample. Bromphenol blue is used as an indicator, and a bluish-green color is produced on the surface of the tablet in the presence of albumin. The increased sensitivity of the Microbumintest results over those of Albustix is attributable to the different test procedures. Instead of immersing a test strip into the sample, as with Albustix, a drop of urine is placed in the center of the tablet. After the albumin contained in the sample is completely absorbed into the tablet, excess urine is washed away with 2 drops of water, leaving a relatively higher local concentration of albumin on the tablet.

A sign of "+" or more (corresponding to an albumin concentration of >40 mg/l) were counted as positive.

All tests were performed as according to manufacturers' recommendations, and without prior knowledge of the results from other tests.

To assess interobserver variation in the reading of colors, we asked 12 nurses from a medical ward to read a tablet or a dipstick test simultaneously, without knowing the readings of one another. Each method was thus tested with standard albumin concentrations of 16, 32, and 64 mg/l.

RESULTS— Urine samples from the 75 patients, assayed with RIA, RID, NEPH, and IT, were found to contain between 0 and 154.2 mg/l of albumin. Only 3 samples had an albumin concentration >50 mg/l. The range of AER calculated from the timed samples was from 0 to 244 μg/min. Taking 20 mg/l of overnight urine albumin excretion as indicative of microalbuminuria (9), 14.6–16%—depending on the method used—of our patients were positive (Table 1).

Correlation coefficients of RID, IT, and NEPH with RIA were 0.970, 0.974, and 0.975, respectively (Fig. 1). Intra- and interassay CVs of the 3 quantitative methods were not significantly different. At low albumin concentration ranges (10–16 mg/l), the intra-assay CVs for RID, IT, and NEPH were 2.5, 6.6, and 11.5%; the interassay CVs were 6.8, 11.4, and 11.5%, respectively. At high albumin concentration ranges (90–120 mg/l) the intra-assay CVs for RID, IT, and NEPH were 1.5, 11.1, and 4.05%, whereas the interassay CVs were 4.5, 5.4, and 1.36%, respectively.

Results of Micral-Test and Microbumintest were compared with RIA in Table 2. Results were positive in 17 of

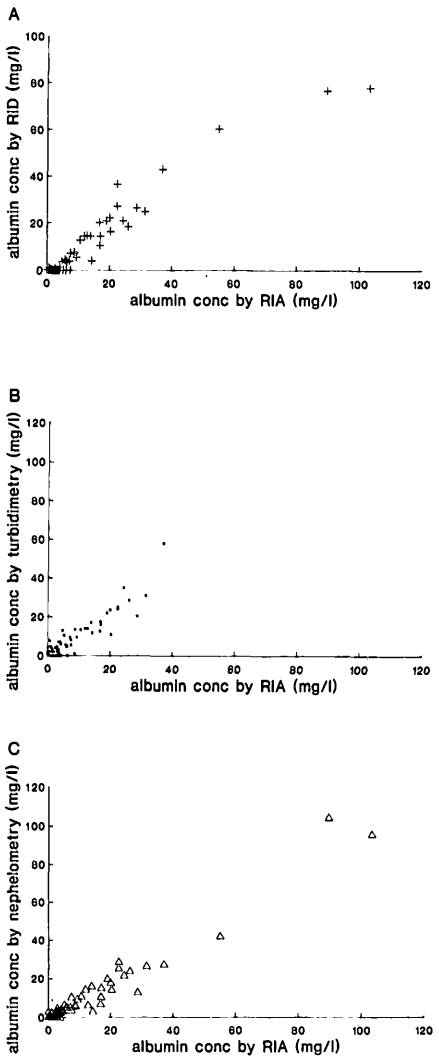

Figure 1—*Correlation of albumin concentration determined by RIA with that of A: RID; B: IT; and C: NEPH.*

75 (22.7%) samples using Micral-Test; 23 of 75 (30.7%) samples were positive by Microbumintest. The Micral-Test sensitivity was 75%, and specificity was 87.3%; respective values for Microbumintest were 100 and 82.5%. In our test patient population, negative predictive values for Micral-Test and Microbumintest were 94.8 and 100%; positive predictive values were 52.9 and 36.4%, respectively.

Simultaneous reading of the colors by 12 nurses accustomed to reading urinary dipsticks showed considerable interobserver variation (Table 3). According to the manufacturer's recommendations, Micral-Test should show either 10 or 20 and Microbumintest should display a negative sign with an albumin concentration of 16 mg/l. All 12 nurses read the tests correctly at this concentration. At a concentration of 32 mg/l, Micral-Test should show either 20 or 50, and Microbumintest should show a negative sign; 2 Micral-Test strips were wrongly read as 100, and 8 Microbumintest tablets were wrongly read as either + or ++. No wrong readings were made with Micral-Test at the albumin concentration of 64 mg/l. Interpretations could not be made with Microbumintest at this concentration because the manufacturer did not quote any concentration ranges for ++.

Table 4 summarizes characteristics of the 7 tests.

Table 2—*Micral-test and Microbumintest testing of 73 urine samples, as compared with RIA*

	Concentration by RIA (mg/L)					
	0-10	10-20	20-30	30-50	50-80	80-130
Micral-test (mg/L)						
100						
50			1		1	2
20	6	2	4	1		
10	15	4				
0	33	3	2	1		
Microbumintest (intensity of color change)						
++					1	2
+	4	7	7	2		
−	33	3	2	1		

Numbers indicate patients showing the particular result.

Table 3—*Interobserver (n-12) variations in the reading of Micral-test and Microbumintest for three albumin concentrations*

	Albumin concentrations (mg/L)		
	16	32	64
Micral test (mg/L)			
100		2	11
50		10	1
20	7		
10	5		
0			
Microbumintest			
++		2	5
+		6	7
−	12	4	

Numbers indicate patients showing the particular result.

CONCLUSIONS— Measurement of microalbuminuria became an important part of diabetic care after it was shown to predict the development of overt diabetic nephropathy (1–3). Moreover, studies (10–12) suggested that treatment of hypertension, low-protein diet, and tight metabolic control at this stage may retard progression to renal failure. Consequently, various techniques were introduced for assaying microalbuminuria (4). This study was done to evaluate which test could best be adopted for our clinical use. We did not examine the usefulness of different urine collection periods (13).

Evaluation of RID, NEPH, and IT against RIA resulted in good and very

Table 4—*Characteristics of RIA, RID, IT, NEPH, Micral-test, and Microbumintest*

	Working range (mg/L)	~Price per test (US $)	Assay time	Equipment required
RIA	0.8–80	2.2	2 h	γ-counter
RID	6–89	3.0	2 days	RID plate-reader
IT	10–160	2.0	1 h	spectrophotometer
NEPH	2–40	4.5	0.5 h	nephelometer
Micral-test	0–>100	1.0	5 min	—
Microbumintest	0–>80	1.0	0.5	—

similar correlations. We chose RIA as the golden standard for comparison because of its well-established reliability in measuring minute quantities of proteins. The intra-assay and interassay CVs of <12% were acceptable; the average intraindividual-biological variation estimated from other studies was >25% (14,15), and the acceptable imprecision of an assay may be defined as ≤50% of the intraindividual-biological variation (16). At urine albumin concentrations of above 50 mg/l, the 4 quantitative assays yielded considerably different results, ranging from 97.8 mg/l for RID to 154.2 mg/l for IT, for an RIA value of 129.5 mg/l in the sample with the highest albumin concentration. However, because we only had 3 samples, no conclusion can be drawn with regard to the reliability of the methods within this range.

NEPH had the advantage of having the widest working range (2–520 mg/L with appropriate dilutions), which is useful for the follow-up of patients with microalbuminuria. Results were rapidly available, within 2 min, and calibration was simple; thus was NEPH cost-efficient even when only small numbers of samples were processed each time. The drawback was that a nephelometer is required, which is relatively expensive.

RID was also convenient for assaying small numbers of samples at one time. An additional advantage is that no sophisticated equipment is required; however, results were not available for 2 days. We believe this is the most practical choice for private clinics with a limited number of patients and technical support, as long as early results are not required.

Turbidimetry was least expensive and reasonably rapid. It also had a wide working range. Like RIA, the manufacturer recommended that more than 10 standards be assayed each time to enable the plotting of a standard curve, which made this test less efficient when assaying only small numbers of samples.

Both turbidimetry and NEPH can be recommended for use by hospital laboratories; the choice depends on the equipment available. RIA, though widely regarded as the golden standard, is less desirable for daily practice because of the rapid deterioration of reagents, and the precautions involved with handling radioactive materials, and the need for a γ-counter.

Qualitative methods are necessarily less accurate. They reflect urinary albumin concentration and are not useful for calculation of the rate of albumin excretion. Yet these tests can be very useful for screening because of their simple procedures, rapid results, and the relatively low costs.

In our evaluation of Micral-Test and Microbumintest, Micral-Test was more specific, which might be expected considering the differences in the underlying principles of these two tests. We were surprised that Microbumintest was more sensitive, because according to the manufacturer its cut-off of + corresponded to concentrations of between 40 and 80 mg/l, which is higher than the Micral-Test + cut-off of ≥20 mg/L. The greater sensitivity of Microbumintest, seems to indicate that it was a better test for screening than Micral-Test. One should note, however, that we and the 12 nurses found it more difficult to match the colors in Microbumintest; and the number of wrong readings was alarming with Microbumintest at the albumin concentration of 32 mg/l. This is important because 32 mg/l is within the concentration range at which albumin excretion becomes microalbuminuric. Therefore, the usefulness of the Microbumintest may be rather observer dependent.

The specificity for Micral-Test was only 87.3%, and was even lower for Microbumintest. We must, therefore, emphasize that these qualitative tests are useful only for preliminary screening. Positive results should always be verified with quantitative assays before making the diagnosis of microalbuminuria.

We attempted in this study to evaluate the advantages and disadvantages of 4 quantitative and 2 qualitative tests in the assay of microalbuminuria. All the tests are commercially available, and therefore are easily available to clinics and laboratories unable to perform in-house assays.

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