Original Article *Ann Clin Biochem* 1992; **29**: 96–100

Assessment of Micral-Test microalbuminuria test strip in the laboratory and in diabetic outpatients

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SUMMARY. We have evaluated a semi-quantitative dry immunochemical screening method (Micral-Test) for the detection of low concentrations of albumin in urine.

The stability of Micral-Test strips on storage was good, especially with regard to temperature, light and humidity. Changes in urine osmolality (urea and creatinine concentration), pH and sodium and potassium concentration did not have a significant analytical effect on the Micral-Test measurement; extremes of temperature altered the rate of colour development. The depth of dipping the strip into the sample and the timing of reading colour development were critical.

We measured the albumin concentration in 184 urine samples from diabetic outpatients by the Micral-Test and by our in-house immunoturbidimetric method; a Micral-Test result of 20 mg/L had a sensitivity of 91% and specificity of 97% to predict a discriminating urine albumin concentration >30 mg/L by the in house method.

The Micral-Test is suitable for use by non-laboratory personnel and is capable of producing analytically acceptable results for use in diabetes clinics and by general practitioners.

Additional key phrases: diabetic nephropathy; urine proteins; method evaluation

The concentration of albumin in the urine (microalbuminuria) is important in the management of diabetes mellitus as a predictor of later proteinuria indicating advancing diabetic nephropathy.[1] The contention that early treatment with blood pressure lowering agents and improved glycaemic control may reduce albumin loss has resulted in increasing use of the measurement of urinary albumin in the management of diabetic patients.[1–4]

A number of radioimmunoassay and immunoturbidimetric methods have been described for the laboratory measurement of low concentrations of urinary albumin.[5] In addition a number of screening procedures have been developed (Albuscreen, Cambridge Life Sciences; Microbumin Test, Miles Laboratories; and more recently a dry immunochemical method, Micral-Test, Boehringer Mannheim) and promoted for use by laboratory, medical and nursing staff.

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In this paper we report the analytical performance of the Micral-Test compared with a laboratory immunoturbidimetric method.

METHODS

Micral-Test (Boehringer Mannheim, Marburg, Germany) has been designed to detect concentrations of urinary albumin in the range 0–100 mg/L by a dry immunochemical method. The test strip is dipped into the urine sample to a specified depth, removed, and then allowed to incubate at room temperature for 5 min. After moving across a buffer pad (pH = 7·0) the urinary albumin combines with a soluble antialbumin–galactosidase conjugate. The albumin–antibody–galactosidase complex and excess conjugate is eluted onwards to an area containing immobilized albumin where excess conjugate is captured. The albumin–antibody–galactosidase complex then moves onto the substrate pad where the galactosidase reacts with chlorphenolred–galactoside causing a colour change from yellow

to red. After incubation at room temperature for 5 min the strip colour pad is compared to colour blocks provided on the product container. Blocks for 0, 10, 20, 50 and 100 mg/L provide a semi-quantitative estimation of albumin concentration.

A test urine was prepared by adding human albumin (20 mg/L) to a patient urine with pH 5·4, osmolality 265 mosmol/kg and containing urea (86 mmol/L), creatinine (1·8 mmol/L), sodium (65 mmol/L) and potassium (16·8 mmol/L). This urine was aliquoted and stored at −9°C for use in all strip evaluations.

Colour development with time
Following the dipping of four Micral-Test strips into the test urine, the colour development was read at 1 min intervals for 10 min after 3 min initial delay.

Urine composition
The composition of the test urine was changed to test separately the effect of increased concentrations of sodium, potassium, creatinine, urea (500, 500, 50, 1000 mmol/L final concentration, respectively) and pH (8·9 and 3·0) on the results obtained using the Micral-Test. Two strips were used in each test urine.

Depth of dipping
Results obtained by dipping a Micral-Test strip into the test urine to the correct position (to the bottom of the blue zone) were compared to results obtained by dipping three strips to half and three quarters up the blotter pad, and to the top of the blue zone.

Temperature
Two Micral-Test strips stored at 0°C, 21°C, 27°C, 30°C, 36°C and 42°C were immediately dipped into the test urine (as described by the manufacturer) and allowed to incubate at room temperature for the recommended time. Test strips stored at the above temperatures were also dipped into the test urine and then incubated at the storage temperature.

Storage
Micral-Test strips were stored with a desiccant at room temperature, both in the dark (eight strips) and in the light (eight strips), for up to 60 days. Strips were also stored in the dark in conditions of high humidity (in a sealed container, above water) for up to 22 h.

Micral-Test recovery
Human albumin was added to a urine with undetectable albumin levels by immunoturbidimetry to give final concentrations of 20, 50, and 100 mg/L corresponding to the colour pads for Micral-Test. The albumin concentrations of these urines were confirmed by immunoturbidimetry and then assayed using a single Micral-Test strip at each concentration.

Laboratory immunoturbidimetric albumin measurement compared to Micral-Test strip
Three laboratory workers (A, B and C) used Micral-Test strips to measure the albumin concentration in 184 diabetic urine samples previously analysed using an immunoturbidimetric method (Cambridge Life Sciences, Cambridge, UK) on a Hitachi 704 analyser (Boehringer Mannheim, Germany).[6] When this assay was established in our laboratory[6] its accuracy was confirmed using standards prepared by adding human albumin to urine to give final concentrations of 0, 25, 50, 100, 200 and 400 mg/L. In routine use this assay gave within-batch coefficients of variation of 1·5% at an albumin concentration of 20 mg/L and 1·1% at 78 mg/L.

RESULTS

Colour development
After Micral-Test strip was dipped into the sample the intensity of colour development continued to increase during the 10 min observation period. Results for one strip are shown in Fig. 1. Similar results were obtained for all strips tested.

Urine composition
Increased concentrations of sodium and potassium had no effect on the level of albumin measured by Micral-Test (20 mg/L) while changes of osmolality (by increasing urea and creatinine concentration) and of pH caused a slight decrease in the measured concentration (<20, but >10 mg/L).

Depth of dipping
Increasing or decreasing the depth of dipping of Micral-Test strip into the sample, respectively, substantially increased or decreased the albumin concentration measured (Table 1).

Temperature
Storing at 21°C, 27°C, 30°C and 36°C for 1 h followed by immediate dipping and colour development at room temperature caused no change in Micral-Test strip response. However, cooling at 0°C caused a decrease (<20, but >10 mg/L) and

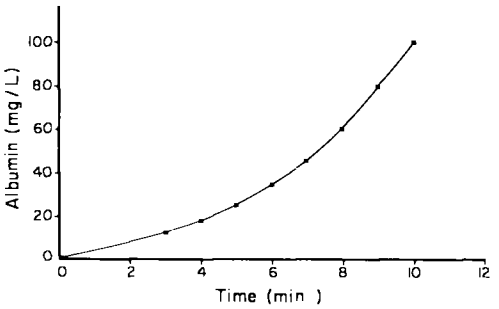

FIGURE 1. *Rate of development of colour, and apparent albumin concentration with time for Micral-Test strip (albumin concentrations were estimated by interpolation between colour blocks).*

TABLE 1. *Effect of depth of dipping Micral-Test into urine on albumin measurement*

Dip depth	Albumin (mg/L)
Correct Position	20
Half way up blotter pad	10
Three quarter way up blotter pad	>10, <20
Top of blue zone	>20, <50 (uneven colour)

heating at 42°C an increase (>20, but <50 mg/L) in the apparent albumin measured.

Incubation at 0°C (<10 mg/L), 36°C and 42°C (both >20, but <50 mg/L) caused changes in the amount of apparent albumin measured while at 21°C, 27°C and 30°C there was no change.

Storage

Storing Micral-Test strips in conditions of high humidity (in the dark) had no effect for up to 6 h, but by 22 h the strip response had fallen markedly (three strips tested). There was no change in Micral-Test strip response to albumin after strip storage at room temperature, both in the dark (four strips tested) and light (four strips tested) for 15 days. However, after 60 days the apparent albumin results obtained decreased markedly for strips stored both in the dark and light.

Micral-Test recovery

The colours developed by Micral-Test strip with urine albumin concentrations of 0, 20, 50, 100 mg/L matched the appropriate colour on the test pads.

Immunoturbidimetric assay recovery

Assay response was linear up to 400 mg/L with recoveries of 100, 100, 100, 105, 100 and 100% at 0, 25, 50, 100, 200, and 400 mg/L albumin, respectively.

TABLE 2. *The distribution of albumin values in 184 urine samples measured by Micral-Test and immunoturbidimetry*

Method	Number of values <20 mg/L	Number of values >20 mg/L
Immunoturbidimetric assay	113	71
Micral-operator A	127	57
Micral-operator B	125	59
Micral-operator C	122	62

Laboratory immunoturbidimetric albumin measurement compared to Micral-Test strip

The 184 urine samples measured by immunoturbidimetry had albumin concentrations which ranged from 0–4110 mg/L with a mean value of 88 mg/L and 71 values greater than 20 mg/L. Of the latter, 40 were in the range 20–100 mg/L, 26 in the range 100–300 mg/L and five greater than 300 mg/L. The distribution of albumin values measured by Micral-Test compared to the values obtained by immunoturbidimetry are shown in Table 2, and the number of true and false positive and negative results for each operator using Micral-Test are shown in Table 3. The corresponding sensitivities, specificities, and positive and negative predictive values for Micral-Test (at 20 mg/L) for discriminating albumin concentrations of 20 mg/L, 25 mg/L and 30 mg/L values (reported as being upper reference intervals by different authors) are also shown. With 20 mg/L as the discriminating albumin concentration the false negative results obtained by Micral-Test strip had values measured by immunoturbidimetry ranging from 21–49 mg/L with a mean value of 33 mg/L.

DISCUSSION

If urine albumin screening methods are to be used in outpatient clinics they must be simple and quick to use, robust, and be able to detect urine albumin concentration with good specificity and sensitivity compared to the radioimmunoassays and immunoturbidimetric methods currently used by laboratories.

In the present study results produced by the Micral-Test method were not influenced by variables such as storage conditions, temperature of reaction and urine composition. However, the most critical variables for strip performance were those most strongly influenced by the operator. In particular, care needs to be taken regarding the depth of dipping the strip into the sample and the timing of reading. The colour intensity increases

TABLE 3. *Comparison of the sensitivity and specificity of Micral-Test at ⩾20 mg/L at various discriminating albumin concentrations determined by immunoturbidimetry*

Albumin cut-off by immunoturbidimetry (mg/L)	Operator	True positives	False negatives	True negatives	False positives	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
	A	71	15	113	1	82·6	99·1	98·6	88·3
20	B	71	15	113	3	82·6	97·4	95·9	88·3
	C	71	12	113	3	85·5	97·4	95·9	90·4
	Mean					83·6	98·0	96·8	89·0
	A	64	6	120	1	91·4	99·2	98·5	95·2
25	B	64	9	120	4	87·7	96·8	94·1	93·0
	C	64	4	120	4	94·1	96·8	94·1	96·8
	Mean					91·1	97·6	95·6	95·0
	A	62	6	122	1	91·2	99·2	98·4	95·3
30	B	62	8	122	5	88·6	96·1	92·5	93·8
	C	62	4	122	4	93·9	96·8	93·9	96·3
	Mean					91·2	97·4	94·9	95·1

rapidly after the recommended reading time such that a delay of 1 min in reading the albumin concentration will cause a positive error of approximately 40%. In addition all users in the present study noted that care had to be taken in reading the 50 mg/L colour pad (brick red) and the 100 mg/L colour pad (wine red) because of colour similarity. We believe that all users need to be educated in the critical aspects of using this technology, as is the case for all rapid diagnostic techniques.

When compared to the laboratory measurement of urine albumin a Micral-Test result of ⩾20 mg/L showed good specificity (that is few false positives), but poor sensitivity (fairly high number of false negatives) when the discriminating albumin concentration was 20 mg/L by immunoturbidimetry. However, by using a Micral-Test result ⩾20 mg/L to predict a discriminating albumin concentration of 30 mg/L (which we believe would be acceptable clinically as a screen) there was a marked improvement in sensitivity with little change in specificity. Similarly, the negative predictive value of Micral-Test improved at an albumin cut off point of 30 mg/L.

The performance of the Micral-Test strip reported in this study compares well with the 96% sensitivity and 88% specificity at 30 mg/L, reported for the Albuscreen latex particle immunoassay screen[7] and with the 97% specificity and 93% sensitivity at a discriminating albumin concentration of 40 mg/L reported for Micro-Bumintest tablets.[8] With Micro-Bumintest the predictive values of a positive test were 89% and 95% at cut off values of 40 mg/L and 30 mg/L, respectively,[8] compared to 95% for Micral-Test at ⩾20 mg/L to predict a cut off of 30 mg/L found in the present study.

A more recent publication has reported numerous false positives with Micro-Bumintest which were unrelated to pH or the presence of blood, both of which have been reported to cause interference in this test.[9] Such results raise the possibility of reaction with non-protein components of the urine, and indeed a study by Jung *et al.*[10] reported that creatinine, uric acid, urea and sodium chloride interfere with the colorimetry of urinary albumin with bromphenol blue, the dye used in Micro-Bumintest test tablets. Antibody-based screening methods such as Micral-Test and Albuscreen should confer an advantage in specificity for albumin.

Large numbers of diabetic patients are at risk of substantial morbidity and mortality associated with diabetic nephropathy.[1,2] With mounting evidence that early detection of increased urinary albumin and clinical intervention may prevent progression to advanced renal disease[3,4,14,15] the measurement of microalbuminuria has become an essential part of the biochemical assessment of these patients. In an earlier study[11] we showed that if a random urine albumin concentration is normal, then the majority of samples over a subsequent 5 year period were also normal, and suggested that a yearly urine albumin test should be carried out in those patients whose initial test is within the normal range. It would seem, from the present study, that Micral-Test is adequate for this screening and could be used by general practitioners, nurses, or small laboratories

without the resources for establishing radioimmunoassays or immunoturbidimetric assays. Those patients with a result outside the normal range could receive further testing either with Micral-Test, or preferably with a laboratory immunoassay to establish the severity of renal disease.

In support of assessing the urinary albumin concentration from a single void urine sample we have noted a good correlation between the first albumin/creatinine ratio determination and the mean of subsequent samples,[11] and between the albumin/creatinine ratio and timed urine collections.[12,13] In addition it has been observed that correction for the urine creatinine concentration adds little to the correlation, only a small improvement in sensitivity.[13] Thus the most appropriate way to use albumin screening methods such as Micral-Test would seem to be with random urine samples.

In summary the Micral-Test appears to provide a robust, sensitive and specific test for clinic screening for increased urinary albumin.

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Accepted for publication 17 July 1991