Multicenter Evaluation of the Micral-Test II Test Strip, an Immunologic Rapid Test for the Detection of Microalbuminuria

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OBJECTIVE — To assess the performance of the Micral-Test II immunologic test strip for the detection of microalbuminuria, a multicenter evaluation in eight European study sites was performed.

RESEARCH DESIGN AND METHODS — Using both the Micral-Test II test strip and the routine method for the determination of albumin concentration, we investigated 2,228 urine samples from diabetic patients. Additionally, interperson variability, color stability, and possible interfering factors (temperature, pH, leucocyturia, erythrocyturia, and drugs) were tested.

RESULTS — For a cutoff concentration of 20 mg/l with respect to the routine methods, a sensitivity of 96.7% and a specificity of 71% were calculated for the Micral-Test II test strip. The negative predictive value was 0.95, and the positive predictive value was 0.78, with a prevalence of positive samples (laboratory method) of 52%. The interperson variability of color interpretation showed 93% concordant readings. The interference study showed an influence of oxytetracycline, leading to higher readings. There was no interference from pH. A sample temperature of <10°C led to lower readings. In the case of samples with massive leucocyturia and erythrocyturia that may delete the chromatographic process, waiting an additional 1–2 min is needed before reading.

CONCLUSIONS — The results of the multicenter evaluation show that the Micral-Test II test strip permits an immediate and reliable semiquantitative determination of low albumin concentrations in urine samples with an almost user-independent color interpretation.

Microalbuminuria is considered as an early marker of nephropathy (1–3). The importance of screening for microalbuminuria, especially in diabetic and to some extent hypertensive patients, is now very well accepted (4,5). The clinical usefulness of the versatile and strong predictive power of microalbuminuria has been further augmented, as it has now been shown that effective intervention modalities exist (6–8).

The possibility of using rapid tests to measure the amount of albumin in urine in the concentration range of importance for microalbuminuria enables the physician to regularly check the albumin status of the urine of his patients (9). This rapid test is of importance in all settings where a fast result is wanted or where there is no access to a quantitative laboratory test (10).

The Micral-Test II test strip, featuring a modification of the detection principle of the Micral-Test test strip, the first immunologic rapid test for microalbuminuria (11), shows the following advantages: based on gold-labeled antibodies, the Micral-Test II test strip is easier to handle, reacts faster, is less timing-dependent, and allows a better color comparison due to the detection principle.

Micral-Test II was evaluated at eight centers in Europe. The results of this multicenter evaluation are reported in the present paper.

RESEARCH DESIGN AND METHODS

Patients and sample material

At the eight centers participating in the study, 2,228 men and women were tested. Patients with type 1 or type 2 diabetes made up the majority of subjects tested; some were hypertensive patients with suspected microalbuminuria. There was no restriction on the sample collection. First and second morning urine samples as well as spot urine samples were included in the study.

Description of the test principle for the Micral-Test II test strip

The Micral-Test II test strip (Boehringer Mannheim, Mannheim, Germany) is a gold-labeled, optically read immunoassay to detect albumin in urine samples. The test-strip architecture is given in Fig. 1. When the test strip is dipped into a urine sample, urine passes via a wick fleece into the conjugate fleece. Any albumin present in the sample binds itself specifically to the gold-labeled antibodies. Excess antibodies are bound by immobilized albumin in the capture matrix. Only antibodies bound to albumin from the urine can pass through. These gold-labeled antibodies flow to the detection pad and turn it red. The reaction

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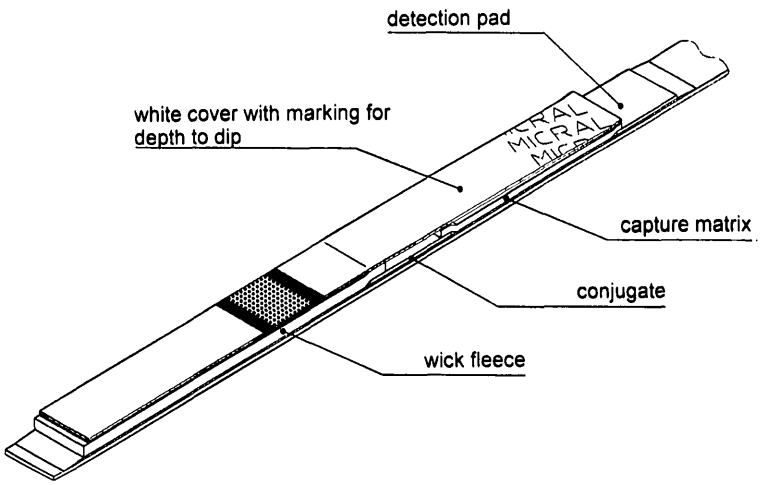

Figure 1—*Architecture of the Micral-Test II test strip.*

time is 1 min. The color is visually compared to color blocks on a chart attached to the vial, with colors representing 0 mg/l, 20 mg/l, 50 mg/l, and 100 mg/l albumin.

Method comparison

Two different lots of the Micral-Test II test strip (lot nos. 296 435 and 284 338) were tested against the routine laboratory method for the determination of urinary albumin used in the eight centers. These methods are given in Table 1. Urine samples were selected such that the number of results with respect to every color block of the Micral-Test II test strip should be the same. The samples were tested for proteinuria using the Combur[10]Test test strip (Boehringer Mannheim). Proteinuric samples found by this measurement were excluded to minimize the number of samples equivalent to the highest color block of the Micral-Test II test strip. The interpretation of the color formed by the test strip was according to the nearest color block. Interpolation of reading results was not allowed. The method comparison for both lots involved both fresh and stored urine samples from diabetic patients. Quality control of the routine methods was performed using five different albumin standards.

Interperson variability

To investigate the reproducibility of the color interpretation of different people in every laboratory, 20 different urine samples, 5 urine samples for each color block, were selected in every laboratory to represent the dynamic range of the Micral-Test II test strip. The reacted strips were interpreted independently by two to five persons in every laboratory. In total, 29 people in seven laboratories were involved.

Color stability

To test the color stability over a 2-week period, 80 already-reacted strips were read repeatedly on several days up to 2 weeks by different people. The reaction color zone was cut off just below the inscription "Micral" to prevent a back-flow of the reaction color due to drying of the test strip. The cut was performed approximately 10 min after the completion of the color development. The color comparison was performed independently of the former reading.

Interference

Pooled urine was spiked with albumin to give concentrations of 2/40/80/160 mg/l.

Table 1—*Results of the quality control experiments at the different evaluation sites*

Evaluation site	Comparison method	*n*	Median (SD/CV) albumin concentration detected at indicated added concentration (mg/l) 10	20	50	100
1	Immunoturbidimetric assay for albumin in urine (Cobas Mira Plus; DAKO)	27	8.79 (0.60/6.9)	16.93 (0.87/5.2)	44.83 (2.23/5.0)	86.97 (4.67/5.3)
2+5	Immunoturbidimetric assay for albumin in urine (antibody from Orion Diagnostika / PEG-Puffer; Hitachi 911 model at 37°C)	17	9 (0.24/2.7)	18 (0.61/3.4)	45 (1.77/4.0)	90 (3.44/3.9)
3	Immunoturbidimetric assay for albumin in urine (Tina-quant MAU Hitachi 704 at 37°C)	6	10.45 (0.24/2.3)	21.45 (1.06/4.9)	50.60 (1.80/3.5)	93.75 (0.78/0.8)
6	Immunoturbidimetric assay for albumin in urine (Tina-quant MAU)	9	12.50 (1.41/11.8)	22.44 (2.60/11.8)	50.27 (3.56/7.1)	92.48 (5.35/5.9)
7	Immunoturbidimetric assay for albumin in urine (in-house test)	10	11.34 (0.55/4.8)	20.32 (0.91/4.4)	46.56 (1.90/4.1)	84.11 (6.26/7.3)
8	BNA-Nephelometer, Behringwerke Marburg, Germany	18	9 (0.73/8.01)	19 (0.86/4.47)	48.5 (3.25/6.76)	94.5 (3.50/3.71)
9	Kinetic nephelometry, Beckman Instruments, Diagnostik Systems, Frankfurt/Main, Germany	24	7.89 (1.35/19.14)	15.60 (0.37/2.40)	42.35 (2.66/6.42)	80.35 (3.06/3.78)
Summary	With respect to the median value of each evaluation site	7	9 (1.63/16.58)	19 (2.46/12.88)	46.5 (3.06/6.54)	90 (5.30/5.97)

Data are medians (mg/l). SD and coefficient of variation (CV) (%) are in parentheses for four different albumin standards provided.

Table 2—*List of drugs tested for interference with the Micral-Test II test strip*

Substance	Concentration (g/l)
Acetylsalicylic acid	1
Ampicillin	1
Ascorbic acid	0.3
Bezafibrat	0.1
Chloramphenicol	0.2
Dopamine	0.01
Glibenclamid	0.001
Methyldopa	0.02
Nicotinic acid	0.1
Oxytetracycline	0.001
Paracetamol	0.2
Phenytoin	0.1
Probenecid	1
Procaine	0.02
Sulfamethoxazole	0.6
Theophylline	0.1
Fosinorm	0.04
Lisinopril	0.04
Dilatrend	0.05

To study possible drug interference, 19 drugs (Table 2) were added to the spiked urine samples at the concentrations given. The possible influence of urine pH and sample temperature was investigated in the same way. Interference was assumed when there was a difference one or more color blocks between the altered sample and the original sample material.

RESULTS

Results of quality control of the routine laboratory method

In Table 1, the results of the quality control are given. The comparison methods were immunoturbidimetric in seven centers and nephelometric in two centers. In all centers, recovery was satisfactory and at a similar level as the interlaboratory variability; thus, the results of the evaluation centers can be pooled.

Method comparison

Urine samples (n = 2,228) were tested with both lots of the Micral-Test II test strip and with the comparison method. For each test-strip lot, the cumulative frequency of reading results of the Micral-Test II test strip against the albumin concentration as measured by the routine laboratory method was calculated separately. For each color block representing a concentration interval on the test vial, a frequency curve was plotted. Comparing the frequency distribution of the two lots, a difference of <3 mg/l albumin was detected. This overall difference is considered to be small for a semiquantitative test relative to the scattering of the laboratory methods. Therefore, an overall plot of the cumulative frequency of the Micral-Test II test strip readings for both lots versus the albumin concentration is given (Fig. 2). This plot demonstrates that the colors given on the vial discriminate between different concentration intervals. It can be seen that the overlapping range between the color blocks is relatively small. For the 10% (maximal sensitivity) and 90% (practical sensitivity) frequencies, separate lines are given in the plot. From this plot, one can deduce the concentration range associated with each color block. The results are given in Table 3. The ranges given for each color block denote the maximum (10th percentile) and the so-called practical sensitivity (90th percentile) of the respective color block.

Eight urine samples with concentrations >200 mg/l were initially found to be nega-

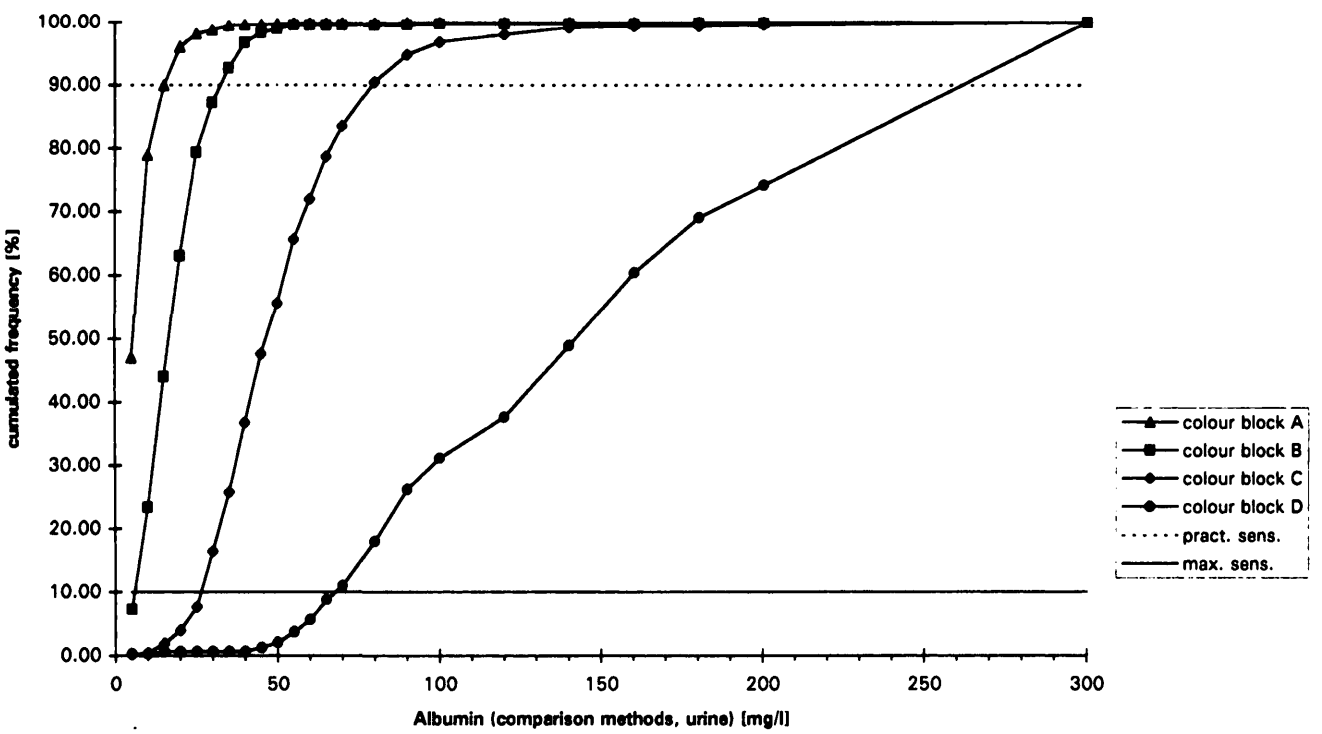

Figure 2—*Summary of the cumulated frequency plots for the Micral-Test II test strip, lot # 284 338 30 and # 296 435 30. Summary of 2,228 pairs of data from the various comparison methods. Shown are the curves for the individual color blocks compared with the values as analyzed by the quantitative methods. Each curve describes the cumulative frequency for test strips assigned to each of the four semiquantitative levels (approximately 0, 20, 50, and 100 mg/l). Dots represents an increase of 5 mg/l (10 mg/l in the range above 70 mg/l) in the quantitative result. The 10% line gives the maximum sensitivity for each color block; the 90% line indicates the practical sensitivity.*

Table 3—*Albumin concentration assigned to the color blocks printed on the label of the Micral-Test II test strip*

Color block	Print on label	Range (mg/l)
A	Negative	0–15
B	~20 (mg/l)	8–35
C	~50 (mg/l)	30–80
D	~100 (mg/l)	70–260

The range gives the values for the 10th and 90th percentiles, also called maximum and practical sensitivity.

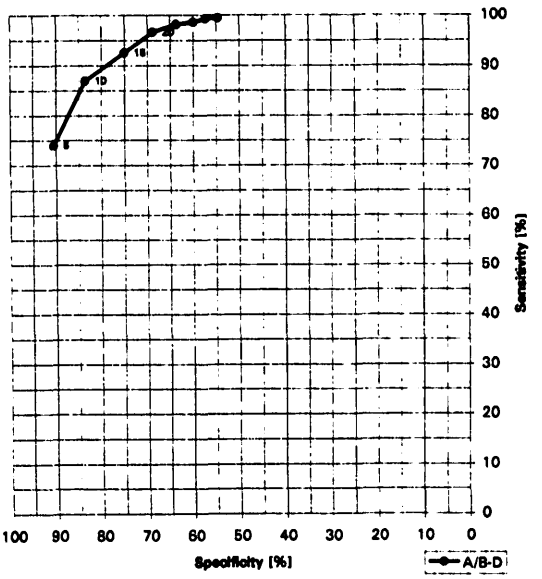

Figure 3—*Receiver operating characteristic plot. Summary of both lots of the Micral-Test II test strip tested. Shown are the results for cutoff values from 5 to 40 mg/l in increments of 5 mg/l. Color block A is assumed to be negative, and color blocks B–D are assumed to be positive.*

tive with the quantitative method (nephelometry) due to the high-dose "hook effect." The same urine samples were classified correctly by Micral-Test II as color block D using the original undiluted sample.

The results of the investigation are presented in Fig. 3 in the form of a receiver operating characteristic plot with different cutoff concentrations for the discrimination between negative (color block A) and positive (color blocks B, C, and D). For a cutoff concentration of 20 mg/l, a sensitivity of 96.7% and a specificity of 71% was calculated. The negative predictive value was 0.95, and the positive predictive value was 0.78, with a 52% prevalence of positive samples (laboratory method).

Interperson variability of the color interpretation of the Micral-Test II test strip

At 7 of the 8 evaluation centers, 20 urine samples, representing the concentration range for the Micral-Test II test strip, were tested by 29 different users. Of 580 possible concordant readings for different urine samples, 538 concordant results (93%) were obtained. In 40 cases (7%), the neighboring color block was assigned to the respective urine sample. In only two cases (0.3%), the difference was two color blocks.

Color stability of the reacted Micral-Test II test strip

Of 80 urine samples tested by different people over a 2-week interval, the reaction color stayed stable on 60% of the cases. In 36% of the cases, a shift by one color block was observed. Only in 4% of the cases was a color shift greater than one color block observed.

Interferences

From the drugs as given in Table 2, only oxytetracycline showed an interference leading to higher readings. Variation of sample pH between 4 and 10 showed no influence. Sample temperatures <10°C led to lower readings. Observations during the course of the external evaluation indicated that massive leucocyturia and erythrocyturia may delete the chromatography process. For these samples, readings should be delayed for an additional 1–2 min.

CONCLUSIONS — The results of the multicenter evaluation of the Micral-Test II test strip show that this test permits a reliable semiquantitative determination of low albumin concentrations in urine samples. These results were obtained by comparing the color of the reaction to the nearest color block. A change in this color interpretation policy, i.e., by downward or upward reading, will influence both sensitivity and specificity. The experiment for the detection of interperson variability demonstrated that the color interpretation is almost user independent.

The reaction of the Micral-Test II test strip takes only 1 min to reach a stable reaction color. Earlier studies showed that readings up to 24 h after testing gave results identical to readings after 1 min (12). The present data show that readings over a 2-week interval gave identical results in 60% of cases and that a shift by one color block was observed in 36%. The reaction colors are clearly distinguished from one another. The color comparison scale for this test differentiates urine samples in good agreement with quantitative urinary albumin tests.

From these results, we see a big advantage for a busy nurse in a clinic or practice to evaluate different urine samples simultaneously for urinary albumin in a series.

In conclusion, the Micral-Test II test strip is a reliable, immediate on-site test. Readings within 24 h, according to previous studies, do not affect the results, whereas readings after longer intervals in this study resulted in deviations and cannot be recommended. The Micral-Test II test strip can be used for screening and monitoring programs as outlined, i.e., in the St. Vincent Declaration, 1994, guidelines for the prevention of diabetic renal failure (13), as well as in several other guidelines recently reviewed (5). It is, of course, not restricted to diabetic renal failure, but also suited for hypertensive and nephrology patients (14,15).

References

1. Borch Johnsen K, Wenzel H, Viberti GC, Mogensen CE: Is screening and intervention for microalbuminuria worthwhile in patients with insulin dependent diabetes? *BMJ* 306:1722–1723, 1993
2. Whiteside CI: Detection of progressive diabetic nephropathy: role of microalbuminuria determination. *Clin Biochem* 26:311–313, 1993
3. Mogensen CE: Proceedings of a meeting on epidemiology of microalbuminuria,

November 13, 1993, Mortefontaine, France. *J Diabet Complications* 8:133–192, 1993
4. The Diabetes Control and Complication Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977–986, 1993
5. Mogensen CE, Keane WF, Bennett PH, Jerums G, Parving HH, Passa P, Steffes MW, Striker GE, Viberti GC: Prevention of diabetic renal disease with special reference to microalbuminuria. *Lancet* 346:1080–1084, 1995
6. Marre M, Chatellier G, Leblanc H, Guyene TT, Menard J, Passa P: Prevention of diabetic nephropathy with enalapril in normotensive diabetics with microalbuminuria. *Br Med J* 297:1092–1095, 1988
7. The Microalbuminuria Captopril Study group: Captopril reduces the risk of nephropathy in insulin-dependent diabetic patients with microalbuminuria. *Diabetologia* 39:587–593, 1996.
8. Mathiesen ER, Hommel E, Giese J, Parving HH: Efficacy of captopril in postponing nephropathy in normotensive insulin dependent diabetic patients with microalbuminuria. *BMJ* 303:81–87, 1991
9. Poulsen PL: Office tests for microalbuminuria. In *The Kidney and Hypertension in Diabetes Mellitus*. Mogensen CE, Ed. Boston, MA, Kluwer, 1994, p. 95–102
10. Le Floch JP, Charles MA, Philippon C, Perlemuter L: Cost-effectiveness of screening for microalbuminuria using immunochemical dipstick tests or laboratory assays in diabetic patients. *Diabet Med* 11:349–356, 1994
11. Hasslacher C: Clinical significance of microalbuminuria and evaluation of the Micral-Test®. *Clin Biochem* 26:283–287, 1993
12. Poulsen PL, Mogensen CE: Evaluation of a new semiquantitative stix for microalbuminuria. *Diabetes Care* 18:732–733, 1995
13. Viberti GC, Mogensen CE, Passa P, Bilous R, Mangili R: St Vincent Declaration, 1994: Guidelines for the prevention of diabetic renal failure. In *The Kidney and Hypertension in Diabetes Mellitus*. Mogensen CE, Ed. Boston, MA, Kluwer, 1994, p. 515–527
14. Biesenbach G, Kaiser W, Grafinger P, Stuby U, Janko O, Zazgornik J: Mikroalbuminurie bei essentieller Hypertonie und normaler Nierenfunktion. *Wiener Medizinische Wochenschr* 142:23–26, 1992
15. Fliser D, Arnold U, Ritz E: Prävalenz der Mikroalbuminurie bei Patienten mit primärer Hypertonie. *Nieren- und Hochdruckkrankheiten* 21:660–661, 1992