

Laboratory Evaluation of Leukocyte Esterase and Nitrite Tests for the Detection of Bacteriuria

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We compared the sensitivity, specificity, and predictive values of the 1-min leukocyte esterase test and the test for urinary nitrite alone and in combination as screening tests for bacteriuria in over 5,000 clinical urine specimens. The leukocyte esterase-nitrite combination had a sensitivity of 79.2%, a specificity of 81%, and a negative predictive value of a negative test of 94.5% for specimens with $\geq 10^5$ CFU/ml. Although the sensitivity of this test was too low to allow its use as the only screening test for bacteriuria, it may serve as a useful adjunct to culturing and other urine-processing systems in the microbiology laboratory.

Many screening methods have been proposed to detect bacteria in urine on the day of collection, both to shorten the delay inherent in the culture system and to decrease the time spent in the laboratory separating negative specimens from those considered to have significant bacteriuria. These screening tests include several manual (4, 5, 8, 13, 15, 18), semiautomated (10, 11, 14), and automated systems (7, 9, 10, 17).

One of the most promising of the many manual, growth-independent tests currently available is the Chemstrip LN (Bio Dynamics, Indianapolis, Ind.). In this rapid (1- to 2-min), inexpensive (\$0.15 per test) screening test, both a test for leukocyte esterase (LE) activity (a host response-specific test) and a test for urinary nitrite production (a bacteria-specific test) are used to predict bacteriuria (1, 4, 5, 15, 18). Several studies have shown improved sensitivity for the detection of bacteriuria at $\geq 10^5$ CFU/ml when both LE and nitrite (one or both positive) are used versus either test alone (5, 18). Because our own preliminary experience with the Chemstrip LN was promising (M. Pfaller and F. Koontz, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, C285, p. 284), we felt that a large-scale evaluation of the Chemstrip LN as a screening test for bacteriuria was warranted. Thus, we compared the sensitivity, specificity, and predictive values of the LE test and the test for urinary nitrite alone and in combination as screening tests for bacteriuria in over 5,000 specimens submitted to the University of Iowa Clinical Microbiology Laboratory.

A total of 5,218 urine specimens were collected over a 5-month period in sterile containers and processed immediately upon receipt in the laboratory or were stored at 4°C until being processed. All specimens were simultaneously screened with the LN strip and cultured within 2 h of receipt. A 0.001-ml calibrated bacteriologic loop was used to inoculate the urine specimens onto tryptic soy agar with 5% defibrinated sheep blood and eosin-methylene blue agar plates (GIBCO Diagnostics, Madison, Wis.). Colony counts were determined after incubation at 35°C for 24 h, and bacterial and fungal isolates were identified by conventional procedures. Growth was recorded as $<10^3$, 10^3 to $<10^4$, 10^4 to $<10^5$, or $\geq 10^5$ CFU of one or two potential pathogens per ml. Urine specimens containing $\geq 10^5$ or $<10^5$ CFU of nonpathogens per ml (lactobacilli, diphtheroids, *Staphylo-*

ccus epidermidis, and *Streptococcus* spp. other than group D) or multiple (three or more) gram-negative organisms were recorded as such.

The Chemstrip LN test strips were used according to the instructions of the manufacturer. In screening urine specimens with the LN strip, a trace or greater reaction for either LE or nitrite or both was considered positive. The tests were read at 60 and 120 s for LE and at 30 s for nitrite. The readings for the individual tests (LE and nitrite) were recorded for each urine specimen. As recommended by the manufacturer, all previously refrigerated specimens were allowed to come to room temperature (20 min at 25°C) before testing. Specimens that produced staining of the test strips due to urinary pigments were excluded.

Statistical analysis was performed by chi-square testing with the Yates correction (6). The sensitivity, specificity, and predictive values were calculated for the screening tests as previously reported (11).

Of the 5,218 specimens processed during the study, 95 (1.8%) could not be analyzed by the LN strip owing to interfering pigments. The remaining 5,123 specimens were processed as described above. A total of 1,392 specimens (27.2%) contained $\geq 10^5$ CFU/ml, 649 (12.7%) contained $<10^5$ CFU/ml, and 3,082 (60.1%) were sterile. There were 1,228 specimens (24%) containing $\geq 10^3$ CFU of one or two potential pathogens per ml, including 106 with 10^3 to $<10^4$ CFU/ml, 180 with 10^4 to $<10^5$ CFU/ml, and 942 with $\geq 10^5$ CFU/ml.

The results of screening the urine specimens with the LE and nitrite tests are summarized in Table 1. In considering each test singly, the LE test correctly detected 642 (68.2%) of 942 specimens with $\geq 10^5$ CFU/ml, 723 (64.4%) of 1,122 specimens with $\geq 10^4$ CFU/ml, and 760 (61.9%) of 1,228 specimens with $\geq 10^3$ CFU of one or two potential pathogens per ml, whereas the nitrite test detected 422 (44.9%), 440 (39.2%), and 445 (36.2%), respectively ($P < 0.005$ for the comparison of LE versus nitrite at each breakpoint). The nitrite test classified as negative significantly more of the 4,181 specimens containing either contaminants (multiple organisms or nonpathogens present at $<$ or $\geq 10^5$ CFU/ml) or no detectable bacteria: 4,062 (97.2%) versus 3,433 (82.1%) for the LE test ($P < 0.005$).

The performance of the LE test in tandem with the nitrite test resulted in a screening test combination that was significantly better than either test alone. The LE-nitrite combi-

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TABLE 1. Screening of 5,123 urine specimens with Chemstrip LN

Quantitative culture results (CFU/ml)	No. of specimens with following LE-nitrite result:			
	+/+	+/-	-/+	-/-
>10 ⁵ , 1 or 2 potential pathogens	318	324	104	196
10 ⁴ to <10 ⁵ , 1 or 2 potential pathogens	10	71	8	91
10 ³ to < 10 ⁴ , 1 or 2 potential pathogens	4	33	1	68
≥10 ⁵ multiple organisms or nonpathogens	36	84	12	318
<10 ⁵ , multiple organisms or nonpathogens	2	78	5	278
No growth	21	409	20	2,632

nation (one or both positive) correctly detected 746 (79.2%) of 942 specimens with ≥10⁵ CFU, 835 (74.4%) of 1,122 specimens with ≥10⁴ CFU, and 873 (71.1%) of 1,228 specimens with ≥10³ CFU of one or two potential pathogens per ml ($P < 0.005$ versus LE or nitrite alone).

A tabulation of the gram-negative and gram-positive pathogens present at ≥10⁵ CFU/ml is presented in Table 2. The LE-nitrite combination was significantly more sensitive in detecting gram-negative organisms than gram-positive pathogens. Of the 776 gram-negative organisms present at ≥10⁵ CFU/ml, 643 (82.9%) were detected by the LE-nitrite screening test, whereas only 127 (69.8%) of 182 gram-positive bacteria and *Candida* spp. were detected ($P < 0.005$). It is worth noting that the nitrite test is expected to be negative for enterococci and most yeast species; to some extent this may explain the decreased sensitivity with specimens containing these organisms.

Table 3 is a comparative analysis of the LE and nitrite screening tests alone and in combination at ≥10³, ≥10⁴, and ≥10⁵ CFU of one or two potential pathogens per ml. The positive predictive value was significantly greater for the nitrite test alone than for either LE alone or LE and nitrite in combination: 82.2, 81.3, and 78% at ≥10³, ≥10⁴, and ≥10⁵ CFU/ml, respectively ($P < 0.05$). The LE-nitrite combination had a significantly higher predictive value of a negative test than either test alone at all three levels of bacteriuria ($P < 0.01$).

The results obtained in this study demonstrate that the combination of a host response-specific test (LE) with a bacteria-specific test (nitrite) results in a more sensitive screen for bacteriuria than either test alone (Table 3). This is true at levels of bacteriuria ranging from ≥10³ to ≥10⁵ CFU/ml. More important, when both tests (LE and nitrite) are negative, one can predict with a high degree of confidence (predictive value of a negative test, 94.5%; Table 3) that the urine specimen will contain <10⁵ CFU/ml. These findings are in agreement with those reported in several previous studies (2, 5, 15, 18; H. Nadler, P. Harris, L. Mele, and S. Kurtz, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, C190, p. 268; C. Reichart and K. Heier, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, C195, p. 269), although we found a somewhat lower sensitivity (79.2%) and predictive value of a negative test (94.5%) for the combination at the ≥10⁵ CFU/ml level. It should be emphasized that the predictive values of a positive and a negative screening test will vary depending on the prevalence of bacteriuria (or disease) in the population studied (12). Thus, the test may perform quite differently in a highly selected patient population as com-

pared with the unselected population studied here. This should be taken into account when predictive values are used to justify (or denounce) the use of a screening test in the clinical microbiology laboratory of a specific hospital (2, 3). The decreased sensitivity and predictive value of a negative test in the current study may be a function of multiple factors including the patient population, the prevalence of bacteriuria in the population studied, larger sample size, and the fact that the study was carried out over a 5-month period with testing being performed by multiple technologists rather than one or two individuals.

The rather low sensitivity of the Chemstrip LN for detecting bacteriuria obtained in this study raises serious doubts as to its utility as the only screening test for urine specimens in the clinical laboratory (16). If only those specimens positive by the Chemstrip LN (LE or nitrite or both positive) were cultured, the laboratory would eliminate 69.9% of all the urine cultures sent to the laboratory. Obviously, this would result in considerable savings in materials and labor; however, this savings would be at the expense of missing 20.8% of all of the specimens with ≥10⁵ CFU of one or two potential pathogens per ml and up to 28.9% of those with ≥10³ CFU/ml. In particular, patients with neutropenia or infections with nitrate reductase-negative organisms would be missed with this test.

Despite the above limitations of the Chemstrip LN as a primary urine screen, we feel that there are still several possible uses for this rapid and inexpensive test in the clinical microbiology laboratory. Examples of potential applications include the screening of selected patients at the bedside or in the clinic (3; M. Pfaller, B. Ringenberg, R. Niska, L. Rames, J. Hegeman, and F. Koontz, Abstr. Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 701, 1984), use in tandem with the newer semiautomated urine screening devices such as Lumac (M. Pfaller et al., Diagn. Microbiol. Infect. Dis., in press; L. F. Freundlich and S. E. Perez, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, C284, p. 284), and use in conjunction with a highly automated urine processing system such as the AutoMicrobic system (2; Pfaller and Koontz, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, C285, p. 284).

In conclusion, we feel that the sensitivity of the Chemstrip LN is too low to allow its use as the only screening test for

TABLE 2. Detection of potential pathogens by the LE-nitrite test

Organism	No. of isolates ^a	No. of isolates detected (%)
Gram negative		
<i>Escherichia coli</i>	455	369 (81)
<i>Klebsiella</i> spp.	108	86 (80)
<i>Enterobacter</i> spp.	41	36 (88)
<i>Citrobacter</i> spp.	17	17 (100)
<i>Serratia</i> spp.	3	3 (100)
<i>Salmonella</i> spp.	1	1 (100)
<i>Proteus</i> spp.	48	41 (85)
<i>Providencia</i> spp.	6	6 (100)
<i>Morganella</i> spp.	1	1 (100)
<i>Pseudomonas aeruginosa</i>	96	83 (86)
Gram positive		
<i>Staphylococcus aureus</i>	24	23 (95)
<i>Streptococcus</i> sp.	100	61 (61)
Group D		
<i>Candida</i> spp.	58	43 (74)

^a A total of 958 organisms (≥10⁵ CFU/ml) were isolated in 942 urine specimens.

TABLE 3. Comparative analysis of two screening tests alone and in combination

Screening test	Sensitivity (%) at breakpoint ^a :			Specificity (%) at breakpoint ^a :			Predictive value (%) of test at breakpoint ^a :					
							Positive			Negative		
	10 ³	10 ⁴	10 ⁵	10 ³	10 ⁴	10 ⁵	10 ³	10 ⁴	10 ⁵	10 ³	10 ⁴	10 ⁵
LE	61.9	64.4	68.2	83.8	83.3	82.1	54.7	52.0	46.2	87.5	89.3	92.0
Nitrite	36.2	39.2	44.9	97.5	97.5	97.2	82.2	81.3	78.0	82.9	85.1	88.7
LE-nitrite ^b	71.1	74.4	79.2	82.9	82.4	81.0	56.7	54.2	48.4	90.1	92.0	94.5

^a Breakpoints for significant bacteriuria in CFU of one or two potential pathogens per ml.

^b Positive test is when either LE or nitrite or both are positive, and a negative test is when both are negative.

bacteriuria in an unselected population. We have identified several alternative uses as an aid in diagnosing urinary tract infections both in the laboratory and at the bedside. The speed and simplicity of this low-cost test make it easily adaptable to a variety of clinical and laboratory situations. The Chemstrip LN may be useful in conjunction with other rapid testing procedures in the clinical microbiology laboratory or in screening highly selected patient populations.

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