

LEUCOCYTE ESTERASE-NITRITE ACTIVITY AS A RAPID SCREEN FOR SIGNIFICANT BACTERIURIA

YS LIM, MSc and ASMAH ISMAIL BSc (Hons)

Bacteriology Laboratory, Department of Pathology, Sultanah Aminah General Hospital, Johore Baru.

Summary

The efficacy of the leucocyte esterase-nitrite test as a screening procedure to detect significant bacteriuria was evaluated and the findings correlated with culture results. Of the 1195 urine samples examined, 127 (10.6%) positive cultures were found. The test had a sensitivity of 78.7%, specificity of 87.7%, positive predictive value of 56.8% and negative predictive value of 95.2%. It is concluded that the low sensitivity and specificity of the leucocyte esterase-nitrite combination do not make it a cost-effective screen for the patient populations in our hospital setting. It may, however, be useful when used in conjunction with other rapid testing procedures.

Keywords: Rapid screening test, leucocyte esterasuria, nitrituria, pyuria, bacteriuria.

INTRODUCTION

Urine cultures constitute a significant proportion of the specimens processed in most clinical microbiology laboratories. The majority of urine samples received for routine culture in a hospital setting do not contain culturable pathogenic bacteria,^{1,2} although the prevalence of urinary tract infection varies for different patient populations. A rapid and sensitive method for screening out negative urine specimens would therefore benefit the laboratory and patient care by reducing costs and improve response time to clinicians.

The standard method for detecting bacterial urinary tract infections is the quantitative culture of a properly collected urine sample.³ However, during the past several years, a number of techniques have been reported as rapid methods for the detection of significant bacteriuria. These include the measurement of growth as monitored by electrical impedance⁴ and turbidity⁵ or by light-scatter nephelometry,^{6,7,8} automated devices for the detection of bacteria by colorimetry^{8,9} and bacterial ATP,^{10,11,12} detection of growth with nitrite as an indicator,¹³ and the presence of leucocyte esterase (LE) as an indicator of pyuria.^{14,15,16}

The purpose of this study was to evaluate the efficacy of Uristix (Ames Division, Miles Laboratories, Australia), a dipstick designed to determine leucocytes and nitrites in urine, for the detection of significant bacteriuria. This screening test was compared with the results of the semiquantitative conventional culture method.

MATERIALS AND METHODS

Urine specimens

A total of 1195 midstream urine specimens from both inpatient and outpatient populations submitted to the Microbiology Laboratory at the Sultanah Aminah General Hospital were included in the study. Urine specimens were collected in sterile containers and received in the laboratory within 2 hours of collection. All specimens were refrigerated at 4°C upon arrival and were processed within 2 hours of collection.

Semiquantitative culture

A calibrated bacteriological loop was used to inoculate the urine specimens onto blood agar and MacConkey agar plates. Colony counts were determined after incubation at 37°C for 24 hours,³ and bacterial and fungal isolates were identified by conventional procedures.¹⁷ Significant bacteriuria was defined as $\geq 10^5$ CFU of one clearly predominant organism per ml. All cultures with two or more organisms were considered contaminated.

Urine screening with Uristix

The Uristix 4 version was selected and used according to the manufacturer's instructions. The plastic strip was dipped into the urine specimen for 1 s, and the strip was withdrawn over the specimen container rim to remove the excess urine. The nitrite reaction and the LE activity were read after 1 and 2 min,

respectively, in accordance with the colour chart supplied by the manufacturer. A reaction of trace or greater for either the LE or nitrite or both was considered positive. The two other parameters of the test strips, namely, glucose and protein, were not evaluated in this study.

Data analysis

The following values were calculated for the performance of the test in screening for bacteriuria : sensitivity = $TP/(TP+FN)$; specificity = $TN/(TN+FP)$; positive predictive value = $TP/(TP+FP)$; and negative predictive value = $TN/(TN+FN)$, where TP is true positive, TN is true negative, FP is false-positive, and FN is false-negative.

RESULTS

Of the 1195 urine specimens examined, only 127 (10.6%) had colony counts indicative of significant bacteriuria. Isolates from urine samples showing significant bacteriuria are shown in Table 1. The culture results also revealed that 451 specimens (37.7%) were contaminated by two or more organisms whereas 617 specimens (51.6%) had either

no growth or insignificant bacteriuria.

The correlation of LE, nitrite and culture results is shown in Table 2. Of the 127 specimens that showed significant bacteriuria, 100 were positive by either LE or nitrite or both, 48 were positive by LE only, and 8 were positive by nitrite only. Twenty seven specimens that showed significant bacteriuria had LE- and nitrite-negative results. Of the 617 specimens that were negative by culture or showed insignificant bacteriuria, 541 were LE-nitrite negative and 76 were positive by the LE-nitrite screen.

The sensitivity, specificity, and predictive values for predicting significant bacteriuria are shown in Table 3.

DISCUSSION

The gram-negative and gram-positive pathogens isolated at $\geq 10^5$ CFU/ml in this study are presented in Table 1. As expected, *Escherichia coli* was the predominant clinical isolate, followed by *Klebsiella pneumoniae*, *Enterobacter* spp. and *Acinetobacter* spp. The results are similar to those obtained by Wenk *et al.*,¹⁸ Smalley and Dittmann,¹⁹ Pfaller and Koontz,²⁰ Wu *et al.*²¹ and Jones *et al.*²²

TABLE 1
SUMMARY OF POTENTIAL PATHOGENS ISOLATED FROM
SIGNIFICANT BACTERIURIA

Organism	No. of isolates (%)
Gram negative	
<i>Escherichia coli</i>	61 (48.0)
<i>Klebsiella pneumoniae</i>	26 (20.5)
<i>Acinetobacter</i> spp.	7 (5.5)
<i>Enterobacter cloacae</i>	5 (3.9)
<i>Enterobacter agglomerans</i>	5 (3.9)
<i>Proteus mirabilis</i>	5 (3.9)
<i>Pseudomonas</i> spp.	3 (2.4)
<i>Providencia rettgen</i>	2 (1.6)
<i>Enterobacter gergoviae</i>	1 (0.8)
Gram positive	
<i>Staphylococcus saprophyticus</i>	5 (3.9)
<i>Staphylococcus epidermidis</i>	5 (3.9)
<i>Candida albicans</i>	1 (0.8)
<i>Candida tropicalis</i>	1 (0.8)

TABLE 2
LEUCOCYTE ESTERASE-NITRITE
ACTIVITY COMPARED WITH CULTURE
RESULTS

LE-nitrite results	Culture results	
	Positive	Negative
Positive*	100	76
Negative	27	541

* LE or nitrite positive, or both.

TABLE 3
SENSITIVITY, SPECIFICITY, AND
PREDICTIVE VALUES OF THE
LEUCOCYTE ESTERASE-NITRITE
SCREEN

LE-nitrite	Calculation (X 100)	Index (%)
Sensitivity	100/(100+27)	78.7
Specificity	541/(541+76)	87.7
Predictive positive	100/(100+76)	56.8
Predictive negative	541/(541+27)	95.2

The ability of the LE-nitrite screen to predict significant bacteriuria has been shown to vary, with sensitivity values ranging from 69.9 to 97.5%.^{15,16,19,23,24,25,26} The predictive indices of the LE-nitrite screening procedure of this investigation are shown in Table 3. Sensitivity and specificity were 78.7% and 87.7%, respectively, and the predictive positive value was 56.8%, which was lower than that found by Smalley and Dittmann¹⁹ and Wu *et al*²¹ but higher than that reported by other investigators.^{15,20,22,24,27} The predictive negative index in the present study was 95.2% and was comparable to that found in previous studies.^{15,18,19,20,21,22,24,27}

In this investigation, we found 76 specimens which were positive by LE and nitrite but had negative cultures (Table 2). These false-positive results could possibly be due to the presence of antimicrobial agents preventing bacterial growth or attributable to viral, chlamydial, or anaerobic pathogens, as suggested by Sawyer and Stone.¹⁶

The LE-nitrite combination was more

sensitive in detecting gram-negative organisms than gram-positive pathogens. Of the 115 gram-negative organisms present at $\geq 10^5$ CFU/ml, 97 (84.4%) were detected by the LE-nitrite screening test, whereas only 3 (25%) of 12 gram-positive bacteria and *Candida spp.* were detected. This finding is in agreement with that observed by Pfaller and Koontz.²⁰ The nitrite test is expected to be negative for gram-positive organisms, notably the enterococci and most yeast species, which may explain the decreased sensitivity with specimens containing these organisms.²⁰ High levels of protein which have been shown to inhibit LE detection²¹ also contributed to the false-negative results in these samples.

It is interesting to note that the LE test performed almost as well as the combined LE and nitrite test. Forty eight specimens were positive by LE only whereas 8 specimens were positive by nitrite alone. This implies that pyuria is a better indicator of significant bacteriuria than nitrituria, as was also observed by other investigators.^{9,22}

We conclude from our study that the LE-nitrite test has a rather low sensitivity and specificity to be useful in our patient populations. The major contribution of a urine screening procedure is its ability to screen out clinically insignificant urine samples rapidly, thus directly reducing the number of urine specimens to be cultured. However, the LE-nitrite screen yielded high numbers of false-negative results which would have a direct impact on those patients whose infections were missed by the test. Also, the low positive predictive value obtained reduces the cost-effectiveness of the test. The LE-nitrite assay may, however, be useful when used in conjunction with other rapid testing procedures in the clinical microbiology laboratory.

ACKNOWLEDGEMENTS

We thank the Director General of Health, Malaysia, for permission to publish this paper. We also thank Mr. E. H. Ng for typing the manuscript.

REFERENCES

- Alexander MK, Khan MS, Dow CS. Rapid screening for bacteriuria using a particle counter pulse-height analyser, and computer. *J Clin Pathol* 1981; 34: 194-8.
- Kelly MT, Balfour LC. Evaluation and optimization of urine screening by Autobac. *J Clin Microbiol* 1981; 13: 677-80.

3. Clarridge JE, Pezzlo MT, Vosti KL. Cumitech 2A, Laboratory diagnosis of urinary tract infections. Coordinating ed. Weissfeld AS. American Society for Microbiology, Washington, D. C. 1987.
4. Cady P, Dufour SW, Lawless P, Nunke B, Kraeger SJ. Impedametric screening for bacteriuria. *J Clin Microbiol* 1978; 7: 273-8.
5. Szilagy G, Aning V, Karmen A. Comparative study of two methods for rapid detection of clinically significant bacteriuria. *J Clin Lab Auto* 1983; 3: 117-22.
6. Jenkins RD, Hale DC, Matsen JM. Rapid semiautomated screening and processing of urine specimens. *J Clin Microbiol* 1980; 11: 220-5.
7. Hale DC, Wright DN, Mckie JE, Isenberg HD, Jenkins RD, Matsen JM. Rapid screening for bacteriuria by light scatter photometry (Autobac): a collaborative study. *J Clin Microbiol* 1981; 13: 147-50.
8. Pezzlo MT, Wetkowski MA, Peterson EM, de la Maza LM. Evaluation of a two-minute test for urine screening. *J Clin Microbiol* 1983; 18: 697-701.
9. Wallis C, Melnick JL, Longoria CJ. Colorimetric method for rapid determination of bacteriuria. *J Clin Microbiol* 1981; 14: 342-6.
10. Thore A, Ansehn S, Lundin A, Bergman S. Detection of bacteriuria by luciferase assay of adenosine triphosphate. *J Clin Microbiol* 1975; 1: 1-8.
11. Schifman RB, Wieden M, Brooker J, Chery M, Delduca M, Norgard K, Palen C, Reis N, Swanson J, White J. Bacteriuria screening by direct bioluminescence assay of ATP. *J Clin Microbiol* 1984; 20: 644-8.
12. Kolbeck JC, Padgett RN, Estevez EG, Harrell W. Bioluminescence screening for bacteriuria. *J Clin Microbiol* 1985; 21: 527-30.
13. Monte-Verde D, Nosanchuk JS. The sensitivity and specificity of nitrite testing for bacteriuria. *Lab Med* 1981; 12: 755-7.
14. Kusumi RK, Grover PJ, Kunin CM. Rapid detection of pyuria by leucocyte esterase activity. *J Am Med Assoc* 1981; 245: 1653-5.
15. Perry JL, Matthews JS, Weesner DE. Evaluation of leucocyte esterase activity as a rapid screening technique for bacteriuria. *J Clin Microbiol* 1982; 15: 852-4.
16. Sawyer KP, Stone LL. Evaluation of a leucocyte dip-stick test used for screening urine cultures. *J Clin Microbiol* 1984; 20: 820-1.
17. Lennette EH, Balows A, Hausler Jr. WJ, Shadomy HJ, eds. Manual of clinical microbiology. 4th ed. American Society for Microbiology, Washington, D. C. 1985.
18. Wenk RE, Dutta D, Rudert J, Kim Y, Steinhagen C. Sediment microscopy, nitrituria, and leucocyte esterase as predictors of significant bacteriuria. *J Clin Lab Auto* 1982; 2: 117-21.
19. Smalley DL, Dittmann AN. Use of leucocyte esterase-nitrate activity as predictive assays of significant bacteriuria. *J Clin Microbiol* 1983; 18: 1256-7.
20. Pfaller MA, Koontz FP. Laboratory evaluation of leucocyte esterase and nitrite tests for the detection of bacteriuria. *J Clin Microbiol* 1985; 21: 840-2.
21. Wu TC, Williams EC, Koo SY, MacLowry JD. Evaluation of three bacteriuria screening methods in a clinical research hospital. *J Clin Microbiol* 1985; 21: 796-9.
22. Jones C, MacPherson DW, Stevens DL. Inability of the Chemstrip LN compared with quantitative urine culture to predict significant bacteriuria. *J Clin Microbiol* 1986; 23: 160-2.
23. Hughes JG, Snyder RJ, Washington II JA. An evaluation of a leucocyte esterase-nitrite test strip and a bioluminescence assay for detection of bacteriuria. *Diagn Microbiol Infect Dis* 1975; 3: 139-42.
24. Males BM, Bartholomew WR, Amsterdam D. Leucocyte esterase-nitrite and bioluminescence assays as urine screens. *J Clin Microbiol* 1985; 22: 531-4.
25. Oneson R, Groschel DHM. Leucocyte esterase activity and nitrite test as a rapid screen for significant bacteriuria. *Am J Clin Pathol* 1985; 83: 84-7.
26. Pfaller MA, Scharnweber G, Stewart B, Koontz FP. Improved urine screening using a combination of leucocyte esterase and the Lumac system. *Diagn Microbiol Infect Dis* 1985; 3: 243-50.
27. Pezzlo MT, Wetkowski MA, Peterson EM, de la Maza LM. Detection of bacteriuria and pyuria within two minutes. *J Clin Microbiol* 1985; 21: 578-81.
28. Smalley DL, Doyle VR, Duckworth JK. Correlation of leucocyte esterase detection and the presence of leucocytes in body fluids. *Am J Med Technol* 1982; 48: 135-7.