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Bacteriuria Screening by Direct Bioluminescence Assay of ATP

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A direct bioluminescence assay for bacteriuria screening is described and compared with the MS-2 system (Abbott Laboratories, Irvine, Tex.) and the chemical strip, Gram smear, and calibrated-loop methods. A total of 973 specimens were tested. Unlike previously described bioluminescence methods, this test measures total ATP in urine without pretreatment of samples to remove somatic ATP. The result was compared with an ATP standard (20 ng/ml). A low result (<3% of standard) was interpreted as negative and a high result (>10% of standard) as positive. Samples with intermediate results (38% of total) were incubated at 35°C in thioglycolate broth (1:10). A 2% increase in ATP concentration was interpreted as positive. The sensitivity of this method for detecting $>10^5$ pathogens per ml was 92.3% and was comparable to those of the MS-2 system (92.7%) and the Gram smear method (90.5%). The chemical strip method was less sensitive (84.0%). The direct bioluminescence method was more sensitive than were the MS-2 system and the Gram smear method for detecting low-level bacteriuria ($<10^3$ to 10^5 organisms per ml), primarily because of associated pyuria. Thioglycolate broth provided a suitable medium for ATP production, and 5% CO_2 decreased bacterial ATP synthesis during log-phase growth. The direct bioluminescence assay is rapid, simple, cost-effective, and reliable for bacteriuria screening.

The standard method for detecting bacterial urinary tract infections is quantitative culture of a properly collected urine sample (1). Other tests that complement this standard method by providing rapid, preliminary results have been described (7). These screening tests are generally employed to quickly identify urine specimens that do not contain substantial numbers of bacterial pathogens and therefore do not require quantitative culture. Likewise, a positive screening test enables the identification of a sample that should be cultured. These screening tests improve the management of patients with suspected urinary tract infections by providing rapid results and help reduce the cost and effort required for processing urine specimens. Previously described screening methods include direct microscopy (17), automated photometric growth detection (3, 15) bacterial ATP measurement by bioluminescence (13), filtration staining (16), and nitrate-leukocyte esterase detection (9). In this report, we describe a bacteriuria screening test that directly measures total ATP in urine and compare this method with three other screening tests and a standard quantitative culture method.

MATERIALS AND METHODS

During a 6-month period, 973 urine samples (both clean-catch and catheter collections) were analyzed. All samples were obtained in the morning from both inpatients and outpatients at the Tucson Veterans Administration Medical Center. Grossly bloody samples were excluded. Nineteen samples were not tested by the MS-2 system (Abbott Laboratories, Irvine, Tex.) and Gram smear methods. The chemical strip test was performed on 555 samples.

Culture. Quantitative cultures were performed by inoculating a well-mixed urine sample onto agar plates containing sheep blood, azide, and eosin methylene blue (GIBCO Diagnostics, Madison, Wis.) with a 0.001-ml calibrated loop. An additional 0.025-ml sample was plated onto a blood agar plate to improve the accuracy of quantitating low-level bacteriuria. All plates were incubated aerobically in 5% CO_2 for at least 18 h. Specimens obtained from patients receiving antibiotics were incubated for an additional 24 h. All cultures were quantitated and identified by standard methods (1).

Pure growth or predominant growth of one or two organisms was interpreted as significant (positive for pathogens) unless diptheroids or *Lactobacillus* spp. were identified. More than two different organisms growing in equal numbers was interpreted as contamination (negative for pathogens).

Bioluminescence assay. Total ATP in urine was measured by bioluminescence with the Turner ATP Bacterial Analysis Kit and Photometric System (Turner Designs, Mountain View, Calif.). The Turner photometer is a semiautomated instrument designed to quantitate ATP by detecting light output from the bioluminescent oxidation of luciferin in the presence of ATP and luciferase. The kit contains luciferin-luciferase reagent, a releasing reagent that liberates ATP from bacterial cells, and an ATP standard (ATP $Na_2 \cdot 3H_2O$, 10 μg/ml).

A 0.1-ml urine sample was placed into 0.9 ml of T3300 thioglycolate broth (GIBCO Laboratories, Grand Island, N.Y.). This sample was vortexed and incubated at 35°C in 5% CO_2. A 0.025-ml sample was removed from the broth and placed into 0.05 ml of releasing reagent before incubation and thereafter at 1 and 2 h. ATP was automatically quantitated with the Turner photometer by injecting 0.05 ml of luciferin-luciferase reagent into the sample mixture. The photometer was set for a 5-s delay period and a 5-s integration period. The delay period eliminates analytic inprecision from inconsistent peak light output during the initial reaction. The integral time is set to quantitate the amount of light output after the peak. Light output during this period is proportional to ATP concentration.

A working ATP standard was prepared by adding a 0.025-ml sample of stock ATP standard to 10 ml of thioglycolate broth. A 0.025-ml sample of this working standard (20 ng of ATP per ml) was added to 0.05 ml of releasing reagent and measured with the photometer as described above. This

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TABLE 1. Comparison of sensitivity and specificity of the various methods

Method	% Sensitivity (no. of samples tested) with following quantitative culture result[a]:						% Specificity (no. of samples tested) with quantitative culture result of <1,000/ml
	>100,000/ml		10,000–100,000/ml		1,000–10,000/ml		
	All organisms	Pathogens only	All organisms	Pathogens only	All organisms	Pathogens only	
Bioluminescence							
1-h incubation	88.7 (230)	92.3 (182)	51.4 (70)	59.6 (47)	36.1 (83)	47.8 (23)	62.9 (590)
2-h incubation	91.7 (230)	95.1 (182)	47.1 (70)	55.3 (47)	39.8 (83)	47.8 (23)	63.7 (590)
MS-2 system	89.9 (227)	92.7 (179)	30.9 (68)	40.0 (45)	12.0 (82)	35.3 (23)	97.1 (577)
Chemical strip	85.2 (122)	84.0 (100)	55.6 (45)	62.5 (32)	37.0 (46)	61.5 (13)	62.3 (342)
Gram smear							
Bacteria only	88.3 (223)	90.5 (179)	15.7 (70)	14.9 (47)	9.8 (81)	13.0 (23)	97.8 (580)
Bacteria + leukocytes	92.8 (223)	93.3 (179)	52.9 (70)	63.8 (47)	35.8 (81)	52.2 (23)	69.0 (580)

[a] Percent sensitivity was calculated as (number of true-positive results)/[(number of true-positive results) + (number of false-negative results)].
[b] Percent specificity was calculated as (number of true-negative results)/[(number of true-negative results) + (number of false-positive results)].

result was used to compute ATP concentrations in the urine samples as a percentage of the ATP standard reading.

Chemical strip method. The Chemstrip LN (Biodynamics, Div. of Boehringer Mannheim Biochemicals, Indianapolis, Ind.) was used to detect leukocyte esterase activity and reduction of nitrite to nitrate in urine samples. The chemical strip is placed into a urine sample for 1 s and then removed. The nitrate reaction is read after 30 s and the leukocyte esterase activity after 1 min, in accordance with the instructions and color chart supplied by the manufacturer. The test was interpreted as positive when either nitrite reduction or leukocyte esterase activity was detected.

MS-2 system. The MS-2 automated urine screening test has been described previously (3). Briefly, a 0.1-ml urine sample is placed into an MS-2 urine ampvette containing 1.0 ml of eugonic broth. The ampvette is photometrically analyzed for growth during a 5-h period in the MS-2 analysis module.

Gram smear. Gram smear examinations were performed on well-mixed, unspun urine samples. At least 20 fields were examined microscopically (magnification, ×1,000) for bacteria, yeasts, and leukocytes. The total number of leukocytes counted in 20 fields was quantitated as follows: 0, none seen; 1+, 1 to 2; 2+, 3 to 9; 3+, 10 to 24; 4+, >24. Two different Gram smear interpretations were made for this study. A positive Gram smear (bacteria only) was defined as the presence of at least one organism per 20 oil immersion fields. Another interpretation of the Gram smear (bacteria and leukocytes) was defined as positive when at least one organism or one leukocyte was observed per 20 oil immersion fields.

Effect of media and CO_2. The effect of media and CO_2 on ATP production was studied by the use of eugonic broth (Abbott), tryptic soy broth with yeast extract (GIBCO), Mueller-Hinton broth (GIBCO), thioglycolate broth (GIBCO), oxoid broth (Abbott), Columbia broth (Difco Laboratories, Detroit, Mich.), and brain heart infusion broth (BBL Microbiology Systems, Cockeysville, Md.). All media except brain heart infusion broth, which was prepared from stock powder, were obtained as sterile broths. Four organisms were tested for ATP production in each broth; an enterococcus (clinical isolate), *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, and *Staphylococcus aureus* ATCC 25923.

A 0.025-ml sample of an inoculum of each organism (equal to a McFarland Standard of 0.5) or an ATP standard (20 ng/ml) was placed into four 1-ml samples of each broth. Duplicate cultures were incubated in air plus 5% CO_2 at 35°C. After 1 and 2 h of incubation, a 0.025-ml sample was removed from each well-mixed broth and added to 0.05 ml of releasing reagent for ATP analysis as described above. The results of each duplicate pair were averaged and recorded. Results were calculated as the difference between the ATP concentrations obtained in the first and second readings.

RESULTS

The sensitivities, specificities, and predictive values of all tests are shown in Tables 1 and 2. The specificity of the bioluminescence test was comparable to those of the Gram smear (bacteria and leukocytes) and chemical strip methods. A relatively high rate of false-positive results was observed in these tests, and most were associated with sterile pyuria. However, these tests were more sensitive than were the MS-2 system and Gram smear (bacteria only) method of detecting low-level bacteriuria (10^3 to 10^5 ml). The chemical strip method had the lowest sensitivity and highest rate of false-negative results for numbers of pathogens $>10^5$/ml. The bioluminescence method showed the lowest rate of false-negative results when a 2-h incubation step was employed (5.2% for numbers of pathogens $>10^5$/ml). A summary of all false-negative results is shown in Table 3.

The effect of pyuria on initial bioluminescence readings is shown in Table 4. Only culture-negative samples were analyzed, to eliminate any effect from bacterial ATP. These results demonstrate that initial bioluminescence readings were influenced by pyuria.

Interpretation of bioluminescence readings. Initial negative readings (<3% of ATP standard) were obtained in 156 (16%) of all urine samples tested by bioluminescence. Three (1.9%) of these low readings occurred with samples having $>10^5$ pathogens per ml. The organisms cultured from these specimens were *Pseudomonas aeruginosa*, an enterococcus, and coagulase-negative *Staphylococcus* spp. All three specimens contained leukocytes, and two showed bacteria on Gram smear. The initial false-negative reading obtained from the sample containing *P. aeruginosa* was 2.9% of the ATP standard and increased to 5.6% after 1 h of incubation.

TABLE 2. Predictive value of all tests compared

Quantitative culture result	% Predictive value of following test[a]:					
	Bioluminescence		MS-2 system	Chemical strip	Gram smear	
	1-h inc.	2-h inc.			Bacteria only	Bacteria + WBCs
Positive (>100,000/ml)						
All organisms	41.7	43.0	81.0	37.8	86.0	45.7
Pathogens only	34.4	35.2	65.9	30.5	69.9	36.4
Negative (<100,000/ml)						
All organisms	94.6	96.1	96.7	93.6	96.4	96.8
Pathogens only	97.1	98.1	98.1	94.3	97.7	97.6

[a] Percent predictive values were calculated as follows: positive by quantitative culture (>100,000 organisms per ml), (number of true-positive results)/[(number of true-positive results) + (number of false-positive results)]; negative by quantitative culture (<100,000 organisms per ml), (number of true-negative results)/[(number of true-negative results) + (number of false-negative results)]. Abbreviations: inc., incubation; WBCs, leukocytes.

A high initial reading (>10% of ATP standard) was seen in 443 (45.5%) of all samples tested. Pathogens were detected in 188 (42.4%) of these samples. In 142 (55.7%) of the remaining samples, leukocytes were seen on Gram smear or nonpathogens were cultured.

Intermediate initial readings (3 to 10% of ATP standard) occurred in 374 (38.4%) of all specimens tested. These samples required incubation and repeat testing for interpretation. A specimen was identified as positive when the bioluminescence reading increased over the base line by 2% of the ATP standard after incubation. A significant rise in ATP concentration was seen in 46 (12.3%) and 48 (12.8%) specimens after 1 and 2 h of incubation, respectively. False-negative results occurred in 11 (2.9%) and 6 (1.6%) specimens containing $>10^5$ pathogens per ml after 1 and 2 h of incubation, respectively. False-positive results were seen in 27 (7.2%) and 26 (7.0%) samples that were culture negative or contained nonpathogens after 1 and 2 h of incubation, respectively.

The effects of different broth media on ATP production are shown in Table 5. Incubation in 5% CO_2 consistently resulted in less ATP production than that seen in non-CO_2 incubation.

DISCUSSION

Bacteriuria screening tests are primarily employed to identify urine samples that do not contain significant numbers of bacterial pathogens. A negative screening test provides valuable clinical information and, in most cases, eliminates the need for quantitative culture. The three general techniques that have been employed for screening are direct staining (16, 17), growth detection (3, 8, 15), and measurement of bacterial byproducts (4, 6, 9).

We evaluated a direct bioluminescence method that combines ATP measurement with growth detection. This method is sensitive for the presence of leukocytes and bacteria in urine. In addition, other cellular constituents (e.g., erythrocytes and epithelial cells) may contribute to the total ATP concentration in urine. Previously described bioluminescence methods attempt to measure only bacterial ATP by selectively removing somatic ATP with detergent and ATPase (14). Somatic ATP has been considered an obstacle to screening urine samples by bioluminescence because of the high rate of false-positive results (13). The false-negative rate, however, should not be influenced by somatic ATP. As expected, there was a relatively high number of false-positive results with the direct bioluminescence test, as well as the chemical strip and Gram smear (bacteria and leukocytes) methods, which are all sensitive to pyuria. The false-positive rate of the bioluminescence test would decline if somatic ATP were removed, but this step prolongs the procedure and eliminates the ability of the test to detect an important finding (pyuria) associated with urinary tract infections. Furthermore, a positive direct bioluminescence test with a negative culture may nevertheless be an abnormal finding that should be clinically investigated (10).

Urine screening tests are not reliably sensitive below 50,000 bacteria per ml (7, 8). This may be an important limitation, because some urinary tract infections are associated with relatively low levels of bacteria. For example, Stamm et al. (11) found that counts as low as 2,000 organisms per ml were associated with infection. The present

TABLE 3. Summary of false-negative results

Organism (total no. tested)	No. of false-negative results by following test[a]:					No. of false-negative results by chemical strip/total no. tested
	Bioluminescence		MS-2 system	Gram smear		
	1-h inc.	2-h inc.		Bacteria only	Bacteria + WBCs	
Enterobacteriaceae (111)	2	0	1	4	3	5/59
Streptococcus spp. (42)	3	3	3	4	2	7/22
P. aeruginosa (34)	4	4	4	6	4	3/16
Staphylococcus spp. (18)	2	1	2	2	2	0/7
Yeasts (11)	3	1	2	1	1	1/3
Other[b] (2)	0	0	1	0	0	0/2

[a] Results shown are for all methods except chemical strip; MS-2 results missing for one each of *Pseudomonas*, *Proteus*, and enterococci; includes urine samples with more than one pathogen (>100,000 pathogens per ml). Abbreviations: inc., incubation; WBCs, leukocytes.

[b] One *Acinetobacter* and one *Neisseria* sp.

TABLE 4. Comparison of leukocyte count to initial bioluminescence readings in culture-negative specimens

Leukocytes	No. tested	Initial readings (%)[a]
0	404	8.4
1+	106	13.8
2+	33	40.5
3+	12	47.6
4+	25	99.6

[a] Mean of all readings expressed as percentage of ATP standard.

study indicates that screening tests influenced by pyuria are more sensitive for detecting low-level bacteriuria. This improved sensitivity is achieved indirectly by measuring the response of the host to infection. Screening tests affected by pyuria may also be more diagnostically reliable because significant, low-level bacteriuria is often accompanied by pyuria (10–12).

Performance of the direct bioluminescence test compared favorably with the other evaluated methods. The overall false-negative rate of $>10^5$ pathogens per ml was 6.4% of all positive results detected and 1.4% of all samples tested. Only three samples containing $>10^5$ pathogens per ml and leukocytes were negative (<3% of ATP standard) on the initial test. This may have been caused by a technical error or an inhibitory effect from unknown urine constituents on the luciferase reaction. False-negative results were also seen in a small number of samples that required incubation (Table 3). These erroneous results could not be attributed to growth inhibition from antibiotic therapy, since true-positive and false-negative results were obtained on approximately equal percentages of samples from patients receiving antibiotics (32 and 27%, respectively).

The sensitivity and predictive value of the MS-2 system and the chemical strip and Gram smear methods were comparable with those in other published reports (3, 7, 9, 14, 17). However, the Gram smear interpretation was set at a relatively sensitive cutoff level (one organism per 20 oil immersion fields) to achieve comparable results. The sensitivity of the Gram smear method was only 77% (for $>10^5$ pathogens per ml) when a positive result was defined as one or more organisms per oil immersion field. This interpretive cutoff level may be too insensitive. For example, Perry et al. (6) reported a comparable sensitivity (77%) when testing midstream urine samples from female patients. However, other investigators have reported 94 to 97% sensitivity when employing an interpretive cutoff of one organism per oil immersion field. These differences may be related to sample selection or technique. We found that the presence of leukocytes on Gram smear of an unspun urine sample enhances the sensitivity of the test, particularly for low-level bacteriuria. This interpretive approach results in a higher number of false-positive results; however, pyuria in the absence of bacteriuria may be significant and should be clinically evaluated.

Growth detection is an important feature of the direct bioluminescence assay. We therefore tested the effect of different broth media on ATP production. The results demonstrated that different media have a substantial influence on ATP production during incubation at 35°C. Thioglycolate broth provided a suitable medium for ATP production. This effect was observed even with *P. aeruginosa*, which generally does not grow well in this broth. In addition, growth in 5% CO_2 was consistently detrimental to ATP production, independent of the broth medium used. These results suggest that incubation without 5% CO_2 would improve the performance of the bioluminescence assay. Additional work is needed to define the factors that contribute to these observed differences. This is important since bioluminescence techniques are more commonly being applied in clinical microbiology procedures (2, 3a–5).

An important feature of bacteriuria screening is the ability to provide rapid, preliminary results which can have an immediate impact on patient care and reduce the amount of labor required for processing urine specimens. In this study, 62% of all samples tested by the direct bioluminescence test could be interpreted in less than 1 min after the initial reading. Most (75%) of these results were positive and required quantitative culture. Bacteriuria or pyuria was detected in 75% of the samples with positive initial readings. In addition, a negative initial reading was highly reliable for excluding bacteriuria. Most (87%) of the additional samples that showed intermediate results and required incubation were interpreted as negative. In practice, therefore, nearly all (91%, in this study) samples requiring culture would be identified by the initial reading.

Most of the culture-positive specimens that required incubation showed a significant rise in ATP concentration after 1 h. Five additional pathogens (>100,000/ml) were detected after 2 h of incubation. The added delay required to obtain these results may not justify the slight improvement in sensitivity achievable with the 2-h incubation procedure.

The direct cost of reagents and supplies for the direct bioluminescence method is about $0.50 per test. When it is assumed that 38% of all specimens screened require incubation and repeat testing, the total average reagent cost is $0.70 per specimen. About 45 s are required to perform each

TABLE 5. Effect of media and CO_2 on ATP production

Broth medium[a]	Increase in ATP concn (ng/ml) between 1 and 2 h of incubation at 35°C							
	Enterococcus		*P. aeruginosa*		*E. coli*		*S. aureus*	
	Air	5% CO_2*	Air	5% CO_2	Air	5% CO_2	Air	5% CO_2
Eugonic	14.1	11.3	16.5	8.3	27.2	24.8	14.4	10.1
TSB with YE	17.9	15.6	7.8	3.7	45.5	23.4	15.2	7.4
Mueller-Hinton	22.9	19.6	9.8	5.3	30.4	25.1	20.8	9.5
Thioglycolate	20.0	14.6	9.6	9.0	20.8	16.2	21.0	7.6
Oxoid	14.0	12.8	3.9	2.4	17.6	9.2	14.7	5.3
Columbia	23.7	17.7	1.4	0.7	22.7	16.7	16.6	7.8
BHI	35.7	25.1	8.9	3.6	39.7	22.3	26.8	10.1

[a] Abbreviations: TSB, tryptic soy broth; YE, yeast extract; BHI, brain heart infusion broth.

test. Therefore, the average testing time per specimen is 1 min when adjusted for repeat testing. If labor expense is $12.00/h, the overall direct cost is $0.90 per specimen. A similar cost analysis of bacteriuria screening tests was reported by Pezzlo et al. (8) and ranged from $0.47 per specimen for the Gram smear to $3.23 for the AutoMicrobic system. These comparisons are difficult to interpret because of variables unique to each laboratory setting. Nevertheless, the cost of performing the direct bioluminescence screen is comparable to other methods.

In summary, the direct bioluminescence method demonstrated acceptable performance when compared with other bacteriuria screening methods. This test could be reliably used to guide the initial microbiological workup of urine specimens and provide preliminary information for the clinical assessment and management of patients.

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LITERATURE CITED

1. **Barry, A. L., P. B. Smith, and M. Turck.** 1975. Cumitech 2, Laboratory diagnosis of urinary tract infections. Coordinating ed., T. L. Gavan. American Society for Microbiology, Washington, D.C.
2. **Hojer, H., and L. Nilsson.** 1978. Rapid determination of doxycline based on luciferase assay of bacterial adenosine triphosphate. J. Antimicrob. Chemother. **4**:503–508.
3. **McCarthy, L. R., T. L. Gavan, J. Robson, and C. Corlett.** 1982. Evaluation of the MS-2 urine screening method for detection of bacteriuria. J. Clin. Microbiol. **16**:250–252.

3a. **McWalter, P. W.** 1984. Determination of susceptibility of *Staphylococcus aureus* to methicillin by luciferin-luciferase assay of bacterial adenosine triphosphate. J. Appl. Bacteriol. **56**:145–150.

4. **Molin, Ö., L. Nilsson, and S. Ånséhn.** 1983. Rapid detection of bacterial growth in blood cultures by bioluminescent assay of bacterial ATP. J. Clin. Microbiol. **18**:521–525.
5. **Nichols, W. W., G. D. W. Curtis, and H. H. Johnston.** 1982. Analysis of the disagreement between automated bioluminescence-based and culture methods for detecting significant bacteriuria, with proposals for standardizing evaluations of bacteriuria detection methods. J. Clin. Microbiol. **15**:802–809.
6. **Perry, J. L., J. S. Matthews, and D. E. Weesner.** 1982. Evaluation of leukocyte esterase activity as a rapid screening technique for bacteriuria. J. Clin. Microbiol. **15**:852–854.
7. **Pezzlo, M. T.** 1983. Automated methods for detection of bacteriuria. Am. J. Med. **75**:71–78.
8. **Pezzlo, M. T., G. L. Tan, E. M. Peterson, and L. M. de la Maza.** 1982. Screening of urine cultures by three automated systems. J. Clin. Microbiol. **15**:468–474.
9. **Smalley, D. L., and A. N. Dittman.** 1983. Use of leukocyte esterase-nitrate activity as predictive assays of significant bacteriuria. J. Clin. Microbiol. **18**:1256–1257.
10. **Stamm, W. E.** 1983. Measurement of pyuria and its relation to bacteriuria. Am. J. Med. **75**:53–58.
11. **Stamm, W. E., G. W. Counts, K. R. Running, S. Fihn, M. Turck, and K. K. Holmes.** 1982. Diagnosis of coliform injection in acutely dysuria women. N. Engl. J. Med. **307**:463–468.
12. **Stamm, W. E., K. F. Wagner, R. Ansel, E. R. Alexander, M. Turck, G. Counts, and K. K. Holmes.** 1980. Causes of the acute urethral syndrome in women. N. Engl. J. Med. **303**:409–415.
13. **Thore, A., S. Ånséhn, A. Lundin, and S. Bergman.** 1975. Detection of bacteriuria by luciferase assay of adenosine triphosphate. J. Clin. Microbiol. **1**:1–8.
14. **Thore, A., A. Lundin, and S. Ånséhn.** 1983. Firefly luciferase ATP assay as a screening method for bacteriuria. J. Clin. Microbiol. **17**:218–224.
15. **Wadke, M., C. McDonnell, and J. K. Ashton.** 1982. Rapid processing of urine specimens by urine screening and the AutoMicrobic system. J. Clin. Microbiol. **16**:668–672.
16. **Wallis, C., J. L. Melnick, and C. J. Longoria.** 1981. Colorimetric method for rapid determination of bacteriuria. J. Clin. Microbiol. **14**:342–346.
17. **Washington, J. A., C. M. White, M. Laganiere, and L. H. Smith.** 1981. Detection of significant bacteriuria by microscopic examination of the urine. Lab. Med. **12**:294–296.