JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1984, p. 797–801
0095-1137/84/100797-05$02.00/0

Vol. 20, No. 4

Comparison of the Lumac and Monolight Systems for Detection of Bacteriuria by Bioluminescence

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Received 12 April 1984/Accepted 20 June 1984

The development of practical and rapid methods for detection of infectious-disease-producing agents in clinical specimens is the most important current goal of clinical microbiology. Bioluminescence is a technique which is rapid and potentially sensitive enough to detect significant numbers of bacteria in urine specimens. To determine whether bioluminescence is practical and cost effective for routine use, we compared two commercially available instruments and kits, Lumac and Monolight, to standard bacterial cultures on 986 urine specimens. Lumac had an overall 83.7% agreement with cultures, a sensitivity of 92.4%, and a specificity of 79.4%. Monolight had 83.5% agreement with cultures, a sensitivity of 89.1%, and a specificity of 81.8%. There were 13.8% false-positive results and 2.5% false-negative results with both systems. When only potentially significant organisms were included, the false-negative rate was reduced to ca. 1%. Both systems are sufficiently accurate to be recommended for routine use. The cost of bioluminescence is higher than that of bacterial cultures, and bioluminescence may not be cost effective in some laboratories.

It takes from 21 to 48 h before physicians receive antibiotic sensitivity reports on organisms isolated from urine cultures. Antibiotics must often be used empirically and discontinued if the cultures are negative, or changed if the organisms are resistant to the antibiotics being used. Some progress has been made in recent years with development of 5-h urine screens with equipment such as the MS-2 instrument (Abbott Laboratories, North Chicago, Ill.) (4). To be clinically useful, however, results should be available within 1 h or less after arrival of the specimen at the laboratory. Bioluminescence is a technique which fulfills such a time requirement. The principle involved is the use of ATP as an index of biomass, by using the firefly reaction in which luciferin-luciferase emits light in the presence of ATP in proportion to the amount of ATP present. Selective lysing agents make it possible to differentiate between somatic and bacterial cell ATP. First, nonbacterial ATP is released and degraded, then bacterial ATP is released and quantitated. Detection of bacteriuria by bioluminescence is not new (1–3, 5, 9, 11), but instrumentation suitable for routine use in clinical laboratories and reliable reagents have not been available commercially, so that in the past bioluminescence has not been widely used. Recently, newer instrumentation and reagents have become available (6–8, 10, 12). Since a substantial percent of urines (65 to 70) submitted for bacterial culture are negative, potentially a considerable amount of time and effort could be saved if negative urines did not have to be plated. We report the results of studies comparing two commercially available instruments and kits, Lumac from the 3M Company (St. Paul, Minn.) and Monolight from Analytical Luminescence Laboratory, Inc. (San Diego, Calif.). We found both systems to be suitable for routine use, but the Lumac system was simpler and required less time. There may be a problem of cost effectiveness for laboratories considering adopting bioluminescence as a routine procedure.

(This work was presented in part at the 84th Annual Meeting of the American Society for Microbiology, St. Louis, Mo., 4–9 March 1984. [Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, C180, p. 226].)

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MATERIALS AND METHODS

Urine specimens. Samples of clean-catch, midstream urine specimens submitted for bacterial culture to the microbiology laboratories of a county hospital and an army medical center were used. Urines not tested or cultured promptly after arrival at the laboratories were stored at 4°C for no longer than 4 h before being cultured or no longer than 6 h before being tested for bioluminescence.

For the Lumac instrument, 0.025 ml of well-mixed urine was tested directly. For the Monolight instrument, 5 ml of urine was centrifuged at 5,000 × *g* for 5 to 15 min, and the supernatant was poured off and discarded. The inverted end of the tube was blotted, and the sediment was tested.

Bacterial cultures. Standard methods for urine cultures were used. Sheep blood and MacConkey agar biplates were streaked with 0.001 ml of urine by using a calibrated loop for bacterial quantitation. Plates were incubated for 18 to 24 h at 35°C. Specimens which gave positive bioluminescence results but showed no growth at 24 h were incubated an additional 24 h at 35°C before being discarded as negative. Isolates were identified by standard methods.

Bioluminescence: general procedure. (i) Nonbacterial ATP present in the specimen was selectively released by addition of a nonionic detergent. (ii) The nonbacterial ATP was degraded by apyrase, an ATPase. (iii) Bacterial ATP was released by a detergent which also helps to inactivate any remaining apyrase by dilution. (iv) A mixture of luciferin-luciferase was added, and light was produced in proportion to the amount of bacterial ATP present. (v) The amount of light produced was measured in a photometer and recorded in relative light units (RLUs).

Photometers for detection of bioluminescence. The following photometers were used for detection of bioluminescence: Biocounter model 2010 (3M Co.) and Monolight 401 (Analytical Luminescence Laboratory).

Bioluminescence detection kits. A comparison of the two detection kits is shown in Table 1. The procedures of each kit are compared in Table 2.

TABLE 1. Comparison of kits and equipment

Parameter	Requirement for:	
	Lumac	Monolight
Amt of urine required	0.025 ml	5.0 ml
Centrifugation	Not necessary	5,000 × *g*, 5 to 15 min to concentrate bacteria, sediment tested
Inactivation of somatic cell ATP	0.050 ml of NRS-Somase, 25 min at 35°C	0.1 ml of Somalight-Apyrase, 5 to 15 min at 25°C
Bacterial ATP release	NRB added; next step	0.2 ml Extralight, 5-min incubation
ATP measurement	NRB + 0.1 ml of Lumit, automatic addition	Specimen to fresh cuvette; add 0.1 ml of Firelight
Luciferin-luciferase (shelf life, reconstituted)	4 weeks, −20°C	3 days, 4 to 8°C
ATPase (shelf life, reconstituted)	4 weeks, 4 to 8°C	4 weeks, −20°C
ATP standard (shelf life, reconstituted)	4 weeks, −20°C	4 weeks, −20°C
Time required		
per specimen	2.5 min	3.0 min
per 20 specimens	40 min	60 min
Cost of instruments	$8,000.00	$5,981.00
Reagent cost per test (basis, 600/month)	$1.20	$1.40
Power requirement	110/220V	210 to 260 V, 50 watts
Recorder output	4 V	4 V
Injection mode	Automatic or manual	Manual
Temp control	Ambient, 25°C, 30°C, 37°C	Ambient
Sample processing	Semiautomated	Manual
Read out	Digital RLUs	Digital RLUs

(i) Lumac bacteriuria screening kit. The Lumac kit (no. 4631; 3M) contained Lumit, purified luciferin-luciferase; NRS, a somatic cell ATP-releasing agent (a nonionic surfactant); Somase, somatic cell ATP inactivator; NRB, bacterial cell ATP-releasing agent. Lumit buffer, HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid] buffer with EDTA; Bactowash, a disinfectant for reagent dispensers; and an ATP standard (10 μg). All reagents were prepared in sterile pyrogen-free water.

(ii) Monolight bacteriuria screening kit. The Monolight kit (no. 8000, Analytical Luminescence Laboratory) contained Firelight, purified luciferin-luciferase; Somalight, somatic cell ATP-releasing agent; Apyrase, somatic cell ATP inactivator; Firelight buffer, HEPES and an ATP standard (10 μg). All reagents were prepared in pyrogen-free, sterile water.

TABLE 3. Result evaluation

Result category	Colony counts (CFU/ml)		RLUs	
	Lumac	Monolight	Lumac	Monolight
Positive	$\geq 10^4$	$\geq 5 \times 10^4$	≥200	≥20
Negative	$<10^4$	$<5 \times 10^4$	<200	<20
False-positive	$<10^4$	$<5 \times 10^4$	≥200	≥20
False-negative	$\geq 10^4$	$\geq 5 \times 10^4$	<200	<20

Evaluation of results. Bioluminescent results were compared with standard bacterial cultures, and results were evaluated as shown in Table 3 as suggested by the manufacturers.

RESULTS

Daily quality control results were always within the limits. The mean for the Monolight positive control was 20.7 RLUs with a 27.5% coefficient of variation, and for Lumac, the mean was 60,136 RLUs with a 13.8% coefficient of variation. The mean for the Monolight negative control was 0.3 RLUs with a 66% coefficient of variation, and for Lumac, the mean was 22.5 RLUs with a 46% coefficient of variation.

Table 4 lists the bacterial isolates and the frequency with which they were encountered.

Table 5 summarizes the bioluminescence results compared with the bacterial culture results. We tested 986 urine specimens by Lumac and 868 urine specimens by Monolight. We were unable to test 118 urine specimens by Monolight because there was less than the required 5 ml of urine available. Lumac correctly predicted the culture results of 83.7% of the specimens, whereas 13.8% of the results were false-positives and 2.5% were false-negatives. Monolight correctly predicted the culture results 83.6% of the time, produced false-positive results on 13.8% of the specimens, and produced false-negatives on 2.6% of the specimens. Sensitivity for Lumac was 92.4%, and for Monolight, sensitivity was 89.1%. The specificity for Lumac was 79.4%; specificity for Monolight was 81.8%. The overall positive predictive value was 68.9% for Lumac and 61% for Monolight. The negative predictive value was 95.4% for Lumac and 95.9% for Monolight.

Table 6 shows the average RLUs for samples divided into the following categories: true-positives, true-negatives, false-positives, and false-negatives, based on bacterial culture results. There was good separation between each category. The mean of true-positives for Lumac was 27,422, and for false-positives, the mean was 2,441; for Monolight, the mean for positive results was 222, and the mean for false-positives was 140.5. The mean of false-negatives was approximately twice that of the true negatives for both systems: 101.5 and 48.2 for Lumac and 8.1 and 3.0 for Monolight.

False-negative results are more important than false-

TABLE 2. Quality control procedures[a]

Procedure	Purpose	Lumac	Monolight
Dark count	Check for light leaks	Empty cuvette, ≤30 RLU	Not done
Reagent blank	Nonspecific light production by reagents	NRB + Lumit, ≤70 RLU	Not done
Negative control	Ability of somase or apyrase to inactivate ATP	ATP + somase, ≤7,000 RLU	ATP + apyrase, <5 RLU
Positive control	Detection of 1.25 ng of ATP	ATP + Lumit, ≥45,000 RLU	ATP + Firelight 15 to 50 RLU

[a] Performed daily.

TABLE 4. Bacteria isolated from urine specimens

Species or organism	No. of isolates
Escherichia coli	143
Mixed bacteria	115
Staphylococci, coagulase-negative	62
Staphylococci, coagulase-negative and diphtheroid bacilli	28
Mixed gram-negative rods	20
Yeasts	15
Klebsiella pneumoniae	13
Proteus mirabilis	11
Nonhemolytic streptococci	11
Diphtheroids	10
Enterococci	8
Pseudomonas aeruginosa	7
Staphylococcus epidermidis	6
Group B streptococci	5
Staphyloccus aureus	3
Lactobacilli	2
Group D streptococci	2
Acinetobacter calcoaceticus	2
Providencia spp.	1
Enterobacter cloacae	1
Alpha-hemolytic streptococci	1
Citrobacter freundii	1
Bacillus spp.	1

positive results since a patient might not receive needed antibiotics. Table 7 analyzes the false-negative results. A total of 39 specimens gave false-negative results. Of these specimens, four were false-negative by both Lumac and Monolight and were considered potentially significant: *Proteus mirabilis* (65,000 CFU/ml), *Acinetobacter calcoaceticus* (65,000 CFU/ml), *Klebsiella pneumoniae* (60,000 CFU/ml), and *Pseudomonas aeruginosa* (100,000 CFU/ml). Monolight alone had 14 false negatives, 9 of which were considered significant: 4 *E. coli* isolates (100,000 CFU/ml), 1 *P. mirabilis* isolate (100,000 CFU/ml), 1 *A. calcoaceticus* isolate (100,000 CFU/ml), 3 *P. aeruginosa* (100,000 CFU/ml). Lumac on the other hand had seven false-negatives which were considered to be significant: four *E. coli* (11,000 to 50,000 CFU/ml) isolates, one *P. mirabilis* (20,000 CFU/ml) isolate, 1 *Pseudomonas* sp. (33,000 CFU/ml) isolate, and one *K. pneumoniae* (100,000 CFU/ml) isolate.

Instructions with both kits state that testing must be performed within 6 h of sample collection. If there is a delay of over 30 min, the samples must be stored at 4°C. Since laboratories cannot always test specimens within 6 h, we retested a number of the specimens after overnight storage at 4°C. Of 90 specimens tested by Lumac, results changed on 7. Four no longer agreed with culture results; however, three which formerly disagreed now agreed. Of 88 specimens retested by Monolight, five results were different after storage. Two no longer agreed with culture results, and three specimens agreed after storage. Changed results were equally distributed among positives, negatives, false-positives, and false-negatives.

Most patients who have urine cultures also have a routine urinalysis performed. We analyzed the urinalysis results of 67 specimens taken at approximately the same time urine was collected for culture and compared them with culture results. These results are shown in Table 8. Sensitivity of the various tests was as follows: leukocytes, 100%; erythrocytes, 62%; bacteria, 57%; and nitrite, 48%. We determined specificity for the same tests and obtained the following results: leukocytes, 57%; erythrocytes, 80%; bacteria, 89%; and nitrite, 83%.

Since all specimens positive by bioluminescence must be cultured, we did a cost analysis to determine the additional cost of screening by bioluminescence. We realize that actual costs will vary from laboratory to laboratory and it would be wise for laboratories considering bioluminescence to do their own cost analyses. The list price for the Lumac photometer is $8,000, and the list price for the Monolight 401 is $5,981. When only reagent cost and technologist time is considered, we determined the cost of a specimen by bioluminescence

TABLE 5. Comparison of Lumac and Monolight results and culture results

Test and result[a]	Culture result		Test comparison[a]			
	Positive	Negative	% Sensitivity	% Specificity	% Positive predictive value	% Negative predictive value
Lumac						
Positive	302	136	92.4	79.4	68.9	95.4
Negative	25	523				
Monolight						
Positive	188	120	89.1	81.8	61.0	95.9
Negative	22	538				

[a] There was not enough of each specimen to test 118 specimens in the Monolight test.

[b] Sensitivity, true-positive results/(true-positive results + false-negative results); Specificity, true-negative results/(true-negative results + false-positive results); positive predictive value, true-positive results/(true-positive results + false-positive results); and negative predictive value, true-negative results/(true-negative results + false-negative results).

TABLE 6. Average RLUs for various result categories

Result category	RLUs with Lumac			RLUs with monolight		
	Mean	SD	SEM	Mean	SD	SEM
Positive	27,422	42,517	2,492	222.0	152.7	11.3
Negative	48.2	44.3	2.0	3.0	152.7	11.3
False-positive	2,441	7,638	675	140.5	130.0	12.2
False-negative	101.5	41.2	8.2	8.1	5.0	1.1

TABLE 7. Summary of false-negative results

Organism isolated	No. of false-negative results (CFU/ml [range]) by the following test(s):		
	Both Monolight and Lumac tests	Monolight only	Lumac only
E. coli	0	4 (100,000)	4 (11,000–50,000)
P. mirabilis	1 (65,000)	1 (100,000)	1 (20,000)
Gram-negative rods (nonfermenter)	1[a] (65,000)	1[b] (100,000)	1[c] (33,000)
K. pneumoniae	1 (60,000)	0	1 (100,000)
P. aeruginosa	1 (100,000)	3 (100,000)	0
Staphylococcus sp. (not *S. aureus*) and diphtheroids	2 (12,000–30,000)	2 (100,000)	8 (15,000–40,000)
Nonhemolytic streptococci (not enterococci)	0	2 (70,000–100,000)	1 (30,000)
Mixed flora	2 (100,000)	1 (100,000)	0
Lactobacillus sp.	0	0	1 (90,000)

[a] *Acinetobacter calcoaceticus* in both tests.
[b] Monolight *Acinetobacter calcoaceticus*.
[c] *Pseudomonas* sp.

was $2.46, which included the plating of true- and false-positives. This was an additional $1.26 per specimen over the $1.20 cost of our standard cultures. These costs were based on the following: (i) the annual number of tests, 7,200; (ii) the percent of positive tests, 33% (estimate, not results from study); (iii) the cost of culture media, $0.30 (standard biplate with blood agar and MacConkey agar); (iv) the cost of technologist time, $11.00 per h (at 2.5 min per test, $0.18/min); and (v) bioluminescence reagent cost, $1.46 per test (80% reagent efficiency assumed). The list price for the two kits was nearly the same, so we did not separate them. We estimated it took ca. 30 s longer to perform a Monolight test than a Lumac test (3.0 min versus 2.5 min) so a ca. 20% increase in time per test should be included in a cost analysis when Monolight is being considered.

DISCUSSION

Thore et al. (12) recently reviewed eight previous studies done between 1975 and 1979. Table 9 shows a brief summary of that data with our results added.

The earliest studies had the poorest correlation with culture results, probably owing to inconsistent reagent quality. Technically, the Monolight system is a bit more difficult and time consuming since it involves more dilution steps and centrifugation. We could not test 118 specimens by Monolight because of the 5-ml specimen requirement. This may be a problem for laboratories with large outpatient volumes. False-positive results are most likely caused by a variety of factors: anaerobes, nonmicrobial ATP from erythrocytes and leukocytes as well as nonviable organisms caused by antibiotic therapy. We cultured 23 of the specimens anaerobically as well as aerobically. Of these specimens, seven (30%) grew a variety of anaerobes. A total of 104 of the false-positive Lumac specimens and 64 of the Monolight specimens had no aerobic growth. If we assume that ca. 30% of these false-positives contained anaerobes, 32 of the Lumac and 20 of the Monolight false-positive specimens would have contained anaerobes. False-negatives do not appear to be a major problem, for when specimens containing a mixture of *Staphylococcus* sp. and diphtheroids, nonhemolytic (not group D) streptococci, mixed flora, and lactobacilli are eliminated, only four specimens would have been considered false-negative by both systems, nine by Monolight only, and seven by Lumac only. If we consider only counts over 100,000 CFU/ml to be significant, Monolight would have had five false-negatives, and Lumac would have had only two. Each laboratory will have to determine what RLU to use to separate positives from negatives. If the primary task is to screen urines, a high sensitivity is needed, and one will have to contend with more false-positives, but if the primary interest is in diagnosis, a high degree of specificity is needed, and the RLU upper limit for negatives should be increased to reduce false-positives. To help minimize false-positives and false-negatives, it might be well for laboratories adopting bioluminescence as a routine procedure to perform a Gram

TABLE 8. Comparison of urinalysis results and culture results

Urinalysis test and result	Culture result		Test comparison[a]			
	Positive	Negative	% Sensitivity	% Specificity	% Positive predictive value	% Negative predictive value
Leukocytes						
Positive	21	0	100	57	51	100
Negative	20	26				
Erythrocytes						
Positive	13	8	62	80	59	82
Negative	9	37				
Bacteria						
Positive	12	9	57	89	71	82
Negative	5	41				
Nitrite						
Positive	10	11	48	83	56	78
Negative	8	38				

[a] Sensitivity, specificity, positive predictive value, and negative predictive value are defined in footnote of Table 5.

TABLE 9. Summary of studies[a]

Bioluminescence study	% Sensitivity	% Specificity	% Positive predictive value	% Negative predictive value
Thore et al. (12) (8 studies)	73–98	28–92	19–70	83–99
Monolight (present study)	89.1	81.8	61.0	95.9
Lumac (present study)	92.4	79.4	68.9	95.4

[a] Sensitivity, specificity, positive predictive value, and negative predictive value are defined in footnote *b* of Table 5.

stain on the specimen and review the routine urinalysis results. We found the microscopic presence of bacteria was as good a positive predictor as bioluminescence. The presence or absence of leukocytes was also helpful, as was a positive nitrite test. When large numbers of leukocytes are present, and the Gram stain is negative, nonbacterial ATP may not be inactivated, thus giving a false-positive result.

A delay of over 6 h before testing urines does not appear to influence results to a great extent, although additional studies need to be done before delayed testing can be recommended.

In summary, detection of bacteriuria by bioluminescence with the two systems we tested appears to be an accurate method and can be recommended. It may also be a better test of effectiveness of antibiotic therapy, for it may detect ATP of nonviable as well as of viable bacteria. Bioluminescence should contribute to better patient care since physicians can have results within 30 min to 1 h and unnecessary antibiotics may be avoided. Each laboratory, however, will have to determine whether the increase in cost can be justified by better patient care.

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