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Evaluation of Two Bioluminescence-Measuring Instruments, the Turner Design and Lumac Systems, for the Rapid Screening of Urine Specimens

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Two bioluminescence-measuring instruments, the Turner Design and Lumac systems, were compared with a standard plate culture method for their ability to rapidly screen 400 urine specimens. For cultures with <1,000 CFU/ml the Turner Design, with old and new evaluation formulas, gave 6.5 and 50.6% false-positive results, respectively, versus 17.6% at ≥500 relative light units with the Lumac. For cultures which had $>10^5$ CFU/ml the Turner Design gave 39% (old formula) and 14% (new formula) false-negative results compared with 4% at <200 relative light units with the Lumac. The microorganisms most frequently isolated in the false-negative cultures from either system were gram-positive cocci. Predictive values for a positive test at $>10^5$ CFU/ml were 77.4% (old formula) and 35.7% (new formula) for the Turner Design versus only 50% for the Lumac at ≥500 relative light units. Predictive values for a negative test for both instruments were >88% at $>10^5$ CFU/ml. The Turner Design and Lumac systems were 4.0 and 3.7 times as expensive, respectively, as the plate culture method. Although both systems greatly reduce the time required to process urine specimens, their high costs as compared with that of plate culture, their failure to detect many specimens having $>10^5$ CFU of gram-positive cocci per ml, and the numerous false-positives reported by both instruments suggest that additional improvements in the systems are warranted.

The evaluation of urine specimens for microorganisms is the most frequently performed procedure in the routine clinical microbiology laboratory. Because the large majority of urine cultures are negative ($<10^3$ CFU/ml), a method for rapidly screening urine specimens for the presence of significant numbers of bacteria is needed. Several automated instruments and technologies recently marketed can detect significant bacteriuria ($>10^5$ CFU/ml) in as little as 1 or 2 min (18, 21) to 6.1 h (8, 15, 17). These systems or methods offer dramatic reductions in the time required to identify positive urine specimens compared with the traditional overnight culture method with one or more plated media.

A relatively new approach to determining the presence of bacteria in urine specimens in the clinical microbiology laboratory has been through the use of instruments able to quantitate bioluminescence. This is accomplished by correlating the number of microorganisms with the quantity of ATP liberated from them after exposure to appropriate nucleotide-releasing agents (23). The quantity of free microorganism ATP is then measured by luciferin-luciferase bioluminescence (23). Instruments measuring bioluminescence also may potentially be used for a large variety of non-urine screening functions such as the rapid detection of bacteria in sterile body fluids (2) and rapid antimicrobial susceptibility testing (24) as well as determination of granulocyte function and numerous biochemical tests relevant to clinical chemistry (25). We report here an evaluation of two bioluminescence-measuring instruments, the Turner Design and Lumac (3M Co.) systems, for the rapid screening of urine specimens.

MATERIALS AND METHODS

Specimens. Four hundred clean, voided urine specimens from inpatients, emergency room patients, and outpatients at Parkland Memorial Hospital were studied. Specimens were refrigerated at 4°C immediately after collection and were processed within 5 h.

Reference procedure. A semiquantitative procedure was used as the reference method (1). Briefly, with a calibrated platinum loop, 0.001 ml of a mixed urine specimen was inoculated onto a 5% sheep blood agar plate and incubated at 35°C aerobically overnight. The plates were then examined for the number and types of organisms present. Most isolates were identified by standard procedures, (3, 5, 9, 11, 14, 16, 20). Some members of the family *Enterobacteriaceae* were identified with a Vitek Systems, Inc., Auto Microbic system 120 instrument. Cultures with three or more organisms were considered mixed.

Lumac and Turner Design procedures. For the Lumac system, 0.025 ml of a mixed urine specimen was pipetted into a plastic cuvette to which a mammalian nucleotide-releasing agent and an ATP-destroying enzyme (Somase) were added. This mixture was shaken and incubated at 35°C for 25 min. The cuvette was then placed in the Lumac biocounter, and a bacterial nucleotide-releasing agent was added. After 10 s, the luciferin-luciferase reagent was added, and the light emitted was measured by a single photon counter with the results expressed as relative light units (RLU). The bacterial nucleotide-releasing agent and luciferin-luciferase reagent were added in a semiautomatic manner by the Lumac biocounter. According to the manufacturer, <200 RLU correlates with $<10^4$ CFU/ml, ≥200 RLU correlates with $>10^4$ CFU/ml, and >500 RLU correlates with $>10^5$ CFU/ml.

With the Turner Design system, the urine sample was diluted 1:10 in nutrient broth, and 1 ml of this mixture was aseptically pipetted into two separate sterile tubes. One tube was incubated at 37°C, and the other was placed in an ice bath. After 1 h 0.025 ml was removed from each tube and placed into separate sterile polypropylene tubes (8 by 50

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TABLE 1. Microorganisms isolated from 400 urine specimens at Parkland Memorial Hospital

Organisms	No. of isolates	
	$>10^4$ to 10^5 CFU/ml	$>10^5$ CFU/ml
E. coli	3	36
Mixed gram-positive cocci	29	18
Mixed gram-positive and gram-negative cocci	3	8
Lactobacilli	7	9
Group B streptococci	0	5
Enterococci	4	5
Proteus mirabilis	0	4
Klebsiella pneumoniae	0	4
Mixed gram-negative rods	0	4
Pseudomonas aeruginosa	1	3
Diptheroids	1	2
Enterobacter colacae	0	2
Gardnerella vaginalis	0	2
Citrobacter freundii	0	2
Staphylococcus epidermidis	11	1
Staphylococcus aureus	0	1
Staphylococcus saprophyticus	0	1
Enterobacter agglomerans	0	1
Yeasts	3	1
Viridans streptococci	3	1
Non-group D enterococci	1	1
Providencia stuartii	1	0

mm) with 0.05 ml of releasing reagent added to each tube. The tubes were vortexed and then placed into the photometer test chamber. Luciferin-luciferase reagent (50 μl) was then added, and the light output was recorded. Two methods of calculating whether a specimen was positive or negative were used. The first, designated as the Turner Design old formula, was as follows: If delta (T_{60} [time, 60 min] light output $-T_0$ [time zero] light output) was greater than the reference number (RN), where

$$RN = \left[\frac{2.8 \times 10^{-9} \text{ g of ATP per ml}}{2.5 \times 10^{-8} \text{ g of ATP per ml}}\right] \times \text{reading of standard}$$

the specimen was considered positive, and if delta was less than or equal to the RN, the specimen was considered negative. The second calculation used in this study, designated as the Turner Design new formula, was determined as follows. If the T_0 reading was 3% or less of the ATP standard, the specimen was considered negative; if the T_0 reading was 30% or more of the ATP standard, the specimen was considered positive. If the T_0 reading was between 3 and 30%, the specimen was considered negative if T_{60} was 10% or less of the ATP standard and delta was 2% or less of the ATP standard, and the specimen was considered positive if T_{60} was 10% or more of the ATP standard and delta was 2% or more of the ATP standard.

TABLE 2. Detection of microorganisms in clinical specimens

Instrument	% Detection[a]	
	$>10^4$ CFU/ml	$>10^5$ CFU/ml
Turner Design		
Old formula	41.1	58.5
New formula	84.4	89.6
Lumac		
≥500 RLU	77.6	94.3
≥200 RLU	87.5	97.2

[a] As compared with detection by plate culture.
[b] When calculated by chi-square analysis (26), these values are significantly different ($P < 0.001$).

TABLE 3. Percentages of false-positive results obtained with the Turner Design and Lumac instruments

Instrument	% False-positive results for the following culture concn:			
	$<10^3$ CFU/ml	10^3 to 10^4 CFU/ml	$<10^4$ CFU/ml	$<10^5$ CFU/ml
Turner Design				
Old formula	6.5[a]	7.0	6.7	9.7
New formula	50.6[a]	63.4	54.6	59.7
Lumac				
≥200 RLU	25.3	52.1	33.3	43.8
≥500 RLU	17.6[a]	31.3	23.3	31.3

[a] When calculated by chi-square analysis (26), these values are significantly different ($P < 0.001$).

Time and cost analysis. A study was conducted to determine the time necessary for evaluation of urine specimens by each system. An analysis was done based on the cost of materials and technologists' time. Technologists' time was calculated based on the average time required to process 25 specimens by each system. The cost of materials was based on the actual costs of purchase for our laboratory. The cost of instruments was not included in this analysis. It should be noted however, that our laboratory makes all our media and that this may result in a lower cost per agar plate than commercially purchased media.

Calculations. Sensitivity, specificity, and predictive values and chi-square analysis were calculated by the methods of Galen and Gambino (6) and Zar (26), respectively.

RESULTS

Detection of microorganisms. Four hundred clean, voided urine specimens were randomly collected. The microorganisms isolated from these specimens are shown in Table 1. Sixty-two urine specimens contained three or more organisms (mixed cultures), and 170 (43%) had less than 10^3 CFU/ml (no growth on a blood agar plate streaked with 0.001 ml of urine). Because mixed cultures may be significant when present in numbers of $>10^5$ CFU/ml per organism (7), and since bioluminescence measures all liberated ATP

TABLE 4. Percentages of false-negative results obtained with the Turner Design and Lumac instruments

Instrument	% False-negative results	
	$>10^4$ CFU/ml	$>10^5$ CFU/ml
Turner Design		
Old formula	47	39[a]
New formula	9	14[a]
Lumac		
<200 RLU	10	4[a]
<500 RLU	19	7

[a] When calculated by chi-square analysis (26), these values are significantly different ($P < 0.001$).

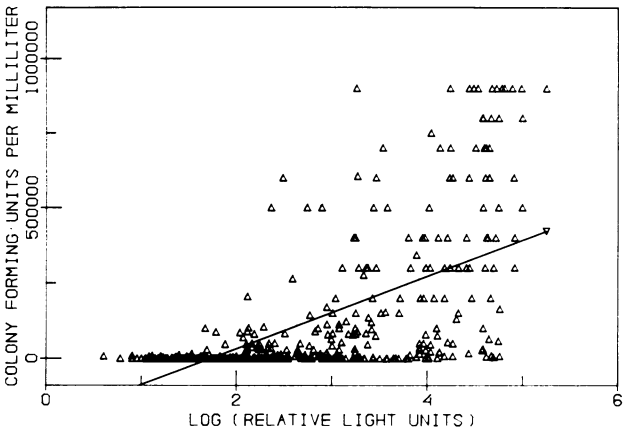

FIG. 1. Correlation between RLU and CFU per milliliter. The correlation coefficient was found to be equal to 0.62 (Spearman's rho). Each triangle represents an RLU reading corresponding to the actual CFU per milliliter found in the urine specimen. Dark triangles indicate multiple readings with similar if not identical values.

whether from a pure or mixed bacterial culture, we included these results in the following determinations. Table 2 shows the ability of the Turner Design and Lumac systems to detect microorganisms in urine specimens. Significant differences in detection were seen between the old and new formula calculations with the Turner Design system: at $>10^5$ CFU/ml, 90% of the specimens were detected with the new formula, contrasted to only 59% detected with the old formula. With an RLU of >500, the Lumac was found to detect 94% of specimens with $>10^5$ CFU/ml.

TABLE 5. Organisms isolated from urine specimens reported as negative by the Turner Design (old formula) system

Concn and organism	No. of isolates
$>10^4$ to 10^5 CFU/ml	
Mixed gram-positive cocci	24
Staphylococcus epidermidis	7
Lactobacillus sp.	7
Yeasts	2
Enterococci	2
Non-group D enterococci	1
Viridans streptococci	1
Diphtheroids	1
Escherichia coli	1
Pseudomonas aeruginosa	1
Proteus mirabilis	1
Providencia stuartii	1
$>10^5$ CFU/ml	
Mixed gram-positive cocci	13
Lactobacillus sp.	5
Group B streptococci	5
Escherichia coli	4
Enterococci	2
Mixed gram-positive cocci, gram-negative rods	2
Viridans streptococci	1
Staphylococcus saprophyticus	1
Staphylococcus epidermidis	1
Non-group D enterococci	1
Enterobacter cloacae	1
Pseudomonas aeruginosa	1
Gardnerella vaginalis	1
Proteus mirabilis	1
Klebsiella pneumoniae	1
Yeasts	1
Mixed gram-negative rods	1

TABLE 6. Organisms isolated from urine specimens reported as negative by the Turner Design (new formula) system

Organism	No. of isolates
$>10^4$ to 10^5 CFU/ml	
Mixed gram-positive cocci	7
Lactobacillus sp.	4
Staphylococcus epidermidis	2
Escherichia coli	1
Pseudomonas aeruginosa	1
Enterococci	1
Non-group D enterococci	1
$>10^5$ CFU/ml	
Group B streptococci	3
Escherichia coli	3
Mixed gram-positive cocci	3
Group D enterococci	2
Viridans streptococci	1
Yeasts	1
Proteus miriablis	1
Staphylococcus epidermidis	1

False-positive and false-negative results. The percentages of false-positive and false-negative urine specimens are shown in Tables 3 and 4, respectively. With sterile cultures, the Turner Design system had 6.5% (old formula) and 51% (new formula) false-positive results. At >500 RLU for the Lumac with sterile cultures, 18% of the results were false-positive. Because of the large number of false-positive results seen with the Lumac, we determined the correlation between RLU and CFU per milliliter (see Fig. 1). The correlation coefficient was found to be 0.62 with Spearman's rho.

The Turner Design system gave 39% (old formula) and 14% (new formula) false-negative results at $>10^5$ CFU/ml.

TABLE 7. Organisms isolated from urine specimens reported as negative by the Lumac instrument

RLU	Organisms isolated (no. of isolates) at the following culture concn:	
	$>10^4$ to 10^5 CFU/ml	$>10^5$ CFU/ml
<500	Mixed gram-positive cocci (9)	Mixed gram-positive cocci
	Staphylococcus epidermidis (2)	*Lactobacillus* sp.
	Yeasts	*Pseudomonas aeruginosa*
	Diptheroids	
	Viridans streptococci	
	Providencia stuartii	
<200	*Lactobacillus* sp. (5)	
	Mixed gram-positive cocci (3)	Group B streptococcus (2)
	Enterococci (2)	*Gardnerella vaginalis*
	Staphylococcus epidermidis (2)	Non-group D enterococci
	Group B streptococci	
	Escherichia coli	
	Mixed gram-negative rods	

TABLE 8. Positive predictive values for the Turner Design and Lumac instruments

Instrument	Positive predictive value (%)[a] for the following culture concn:	
	>10,000 CFU/ml	>100,000 CFU/ml
Turner Design		
Old formula	92.3	77.4[b]
New formula	59.4	35.7[b]
Lumac		
≥200 RLU	67.7	42.4
≥500 RLU	72.7	50.0[b]

[a] Positive predictive value equals the number of true-positives divided by the sum of true-positives plus false-positives.
[b] When calculated by Chi-square analysis (26) these values are significantly different ($P < 0.001$).

The Lumac, at <200 RLU and $>10^5$ CFU/ml, gave 4% false-negative results.

Organisms recovered from urine specimens reported as negative by the Turner Design and Lumac systems. With the Turner Design (old formula) a wide variety of organisms was found in specimens reported as negative (Table 5). The largest number of specimens with $>10^5$ CFU/ml was of mixed gram-positive cocci, followed by lactobacillus and group B streptococci. With the Turner Design (new formula) at $>10^5$ CFU/ml (Table 6), group B streptococci were the most frequently isolated organisms reported as negative, followed by *Escherichia coli* and mixed gram-positive cocci.

With the Lumac at <200 RLU and with urine specimens having $>10^5$ CFU/ml (Table 7), the most frequently isolated organism was the group B streptococcus, followed by *Gardnerella vaginalis* and non-group D enterococcus.

Positive and negative predictive values. The positive predictive values for the Turner Design system at $>10^5$ CFU/ml were 77% (old formula) and 36% (new formula) (Table 8). With the Lumac at $>10^5$ CFU/ml and >200 RLU, the positive predictive value was only 50% (Table 8). Negative predictive values are shown in Table 9. For the Turner Design system at $>10^5$ CFU/ml, the negative predictive values were 88% (old formula) and 97% (new formula). The Lumac showed a negative predictive value of 98% at >500 RLU and $>10^5$ CFU/ml.

Sensitivity and specificity. The sensitivities of the Turner Design system with the old and new formulas at $>10^5$ CFU/ml were found to be 72 and 91%, respectively (Table 10). With the Lumac at <500 RLU and $>10^5$ CFU/ml, the sensitivity was 95% (Table 10). The specificities of the two instruments are shown in Table 11. At $<10^4$ CFU/ml, the Turner Design had specificities of 94% (old formula) and 65% (new formula). The Lumac was found to have a specificity of 82% at >500 RLU and $<10^4$ CFU/ml.

TABLE 9. Negative predictive values for the Turner Design and Lumac instruments

Instrument	Negative predictive value (%)[a] for the following culture concn:	
	>10,000 CFU/ml	>100,000 CFU/ml
Turner Design		
Old formula	68.8	88.4[b]
New formula	88.9	97.0[b]
Lumac		
≥200 RLU	90.9	99.1
≥500 RLU	84.8	98.2[b]

[a] Negative predictive value equals the number of true-negative specimens divided by the sum of true-negatives and false-negatives, times 100.
[b] When calculated by chi-square analysis (26), these values are significantly different ($P < 0.001$).

TABLE 10. Sensitivities of Turner Design and Lumac instruments

Instrument	Sensitivity (%)[a] for the following culture concn:	
	>10,000 CFU/ml	>100,000 CFU/ml
Turner Design		
Old formula	63.8	71.6
New formula	86.5	90.6
Lumac		
<200 RLU	88.9	97.2
<500 RLU	81.7	94.6

[a] False-negative cultures used in the calculation of sensitivity (true-positives divided by the sum of true-positives and false-negatives, times 100) were those that had a RLU count of <200 or <500 RLU or were negative by the Turner Design calculations and were actually positive at the indicated CFU per milliliter.

Time and cost analysis. The cost, working time, and completion time for processing 25 urine specimens by the standard plate method and the Lumac and the Turner Design systems are shown in Table 12. The Turner Design was the most expensive procedure, costing $1.45 per test compared with $1.30 and $0.36 for the Lumac and plate culture methods, respectively. The working times for the plate culture method and Lumac were 30 min each, whereas the Turner Design could take from 5 min (new formula) to 70 min. The completion time was significantly greater for the plate culture method: 18 h versus 30 min for the Lumac and from 5 to 70 min for the Turner Design.

DISCUSSION

The luciferin-luciferase bioluminescence reaction was first described by Dubois in 1885 in *Pyrophorus* (a click beetle) (4). Applications of this ATP-detecting reaction were initially used for measuring the number of bacterial cells via ATP bioluminescence in diverse environments such as aerospace water systems (12) and food products (19). The ATP bioluminescence assay was first used for the detection of bacteriuria by Thore et al. (23), who studied 469 clinical urine specimens and reported that the sensitivity of the assay was sufficient to measure 10^5 CFU/ml. A more automated bioluminescene-measuring instrument developed for bacteriuria screening

TABLE 11. Specificity of the Turner Design and Lumac instruments

Instrument	Specificity (%)[a] for the following culture concn:	
	<10,000 CFU/ml	<100,000 CFU/ml
Turner Design		
Old formula	93.8	91.2
New formula	64.7	62.6
Lumac		
≥200 RLU	75.0	69.6
≥500 RLU	81.8	76.2

[a] Specificity equals the number of true-negatives (all cultures with less than 10^4 or 10^5 CFU/ml) divided by the sum of the true-negatives plus the number of false-positives at the indicated RLU or Turner Design calculation, times 100.

TABLE 12. Cost, working time, and completion time for screening 25 urine specimens

Screening method	Cost of materials ($)	Working time	Completion time	Cost of system ($)
Plate culture[a]	9.00	30 min	18 h	
Lumac system	32.50[b]	30 min	30 min	8,000
Turner Design system	36.25	5–70 min	5–70 min	9,075[c]

[a] All plate culture media were made in Parkland Memorial Hospital. The cost of the plate culture system was essentially that of the materials.
[b] Cost based on reagent rental contract.
[c] Price includes model 20 photometer, syringe injection, and flow cuvette.

was evaluated by Johnson et al. (10) and was found to have a 85% specificity relative to the pour plate technique. Currently there are three commercially available bioluminescence-measuring instruments capable of rapidly screening urine specimens for bacteria in a routine and clinically applicable procedure. They are the Analytical Luminescence Laboratory instrument, the Turner Design instrument, and the Lumac instrument, of which the latter two were evaluated against a standard calibrated loop-plate culture method in the present report.

In our initial studies with the Turner Design instrument, the procedure required a 1-h incubation period before specimen assay in contrast to a total procedure time of 30 min for the Lumac. The Turner Design Corp. then devised a new formula which enabled the screening of urine specimens within 5 min. Although 30% of the urine specimens tested could be rapidly (within 5 min) detected with this new formula, 51% showed false-positive results (Table 3) and 14% showed false-negative results (Table 4). The Turner Design old formula gave significantly fewer false-positive results (6.5%) than did the new formula (51%), versus 18% with the Lumac at >500 RLU. Conversely, the Turner Design old formula gave significantly more (39%) false-negative results than did the new formula (14%) at $>10^5$ CFU/ml. Both calculations for the Turner Design were higher than the percentage of false-negatives seen with the Lumac (4%) at <200 RLU.

In a recent study by Szilagy et al. (22), 25% false-positive (>200 RLU) and 0.25% false-negative (<200 RLU) results were reported with the Lumac after an evaluation of 387 urine specimens compared with a standard plate culture method. However, in their calculations of false-negatives and false-positives, Szilagy et al. used the total number of urine specimens (387), thus including all sterile specimens, instead of the actual number of true-negative or true-positive cultures at the specified CFU concentration. Interpretation of these results and comparison with the data presented here is therefore difficult.

The organisms most frequently isolated from cultures giving Turner Design old or new formula false-negative results (culture positive at $>10^5$ CFU/ml) were gram-positive cocci, including *Staphylococcus epidermidis* and Group B streptococci. This was also true for the Lumac, in which two isolates of group B streptococci and one non-group D enterococcus at concentrations of $>10^5$ CFU/ml had <200 RLU. Mackett et al. (13) also found gram-positive cocci to be the most frequently missed bacteria at $>10^4$ CFU/ml in their evaluation of the Lumac with bacterial strip counts and microscopy. This failure to detect gram-positive cocci may be due to an inability of the bacterial nucleotide-releasing agent to effectively break open the cell walls of these organisms.

The poor predictive value (50%) of the Lumac for specimens with $>10^5$ CFU/ml is the result of the large number of false-positive results obtained with this instrument. A similar explanation accounts for the low positive predictive value with the Turner Design new formula (36%).

The Turner Design and Lumac systems were 4.0 and 3.6 times as expensive as the plate culture method for processing urine specimens. Although both systems offer reduction in completion time from 18 h to 5, 30, or 70 min, the large number of false-positive results obtained with either instrument at $<10^4$ CFU/ml would constitute an additional expense by requiring a plate culture set up when it is not justified (with a criterion of $<10^4$ CFU/ml as being not significant).

In conclusion, both bioluminescence-measuring systems offer significant reductions in the time required to identify positive or negative urine cultures. However, the cost involved, the inability to detect numerous specimens with microorganisms in concentrations of $>10^4$ CFU/ml, and the many false-positive results may reduce their appeal to clinical microbiology laboratories for the rapid screening of urine specimens.

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