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Evaluation of the Lumac kit for the detection of bacteriuria by bioluminescence

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SUMMARY Four hundred and twenty-two urine samples were screened for significant bacteriuria using bioluminescence and microscopy of uncentrifuged urine. A smaller number of false-negatives were seen with bioluminescence (10%) than with microscopy (40%) while both techniques gave a similar number of false-positives (18%). The kit required a large amount of manual preparation, largely pipetting. With this and the short shelf-life of the reconstituted reagents, it is not suitable for small numbers of urines. At 45p per urine, the cost of bioluminescence is too high.

Conventional detection of infected urines is normally achieved by culturing an aliquot and observing growth after overnight incubation. A screening method is needed for urine samples which will detect infected specimens so that direct sensitivity tests can be set up where indicated on the day the specimens are received. Without screening the alternatives are to set up direct sensitivities on all urines (very costly) or on none, thus delaying clinical results. The usual screening test, urine microscopy, is simple, cheap, rapid and effective, considering that it primarily detects pyuria rather than bacteriuria. When large numbers of urines have to be processed microscopy becomes time-consuming.

Bacterial counts can be assessed by measuring bacterial ATP with the bioluminescence luciferin-luciferase technique.[1] This provides a simple, rapid and very sensitive assay for ATP which has been used to detect bacteriuria.[2] However, when applied to the measurement of bacterial ATP in clinical samples such as urine, bioluminescence presents certain problems. Firstly, bioluminescence will detect ATP from both mammalian and microbial sources so that non-bacterial ATP must be released and destroyed before the assay. Secondly, substances present in urine can inhibit the luminescent enzyme reaction.[3] In addition to these problems the methods used for extracting bacterial ATP must be reproducible and have a minimal effect on the luminescent reaction.

Lumac now markets a kit for the detection of bacteriuria in which bacterial ATP is assayed by luciferin-luciferase bioluminescence. Host ATP is extracted from cells and both free and extracted ATP are removed by treatment with the ATP-destroying enzyme apyrase; bacterial ATP is then extracted and, after the addition of luciferin-luciferase reagent, measured by bioluminescence.

We have evaluated the Lumac kit, as marketed, on 422 urines in comparison with routine microscopy against bacterial strip counts, taking into account cost, operator time and ease, as well as efficiency.

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Material and methods

URINE SAMPLES

Four hundred and twenty-two urines sent to the laboratory over a two-week period were used in the study. Normally, the first 40 to 50 urines received each day were used.

MICROSCOPY

This was performed on uncentrifuged urine using a semiquantitative technique.[4] Urines with $\geqslant 200$ WBC/mm^3 were put up on direct sensitivity plates.

VIABLE STRIP COUNTS

Strip counts[5] were done on CLED agar (Lab M) and MacConkey agar (Difco) using Bacteruritest strips (Mast).

IDENTIFICATION OF ISOLATES

Gram-negative organisms were identified by API 20E. Gram-positive bacteria were identified using catalase, coagulase and bile aesculin.

BIOLUMINESCENCE

The Lumac Bacteriuria kit contains luciferin-luciferase reagent (Lumit PM) specific somatic and bacterial nucleotide-releasing agents (NRS and NRB), the ATP-destroying enzyme apyrase (Somase) and a HEPES-based buffer solution (Lumit buffer). Bioluminescence was measured on a Lumac Biocounter, in which light output is measured over the selected time period and expressed as relative light units (RLU).

Five hundred microlitres of urine were mixed with an equal volume of NRS and with 20 μl Somase and incubated at room temperature for 45 min. This released ATP from host cells and destroyed all free ATP. Fifty microlitres of this mixture were then added to 100 μl NRB in the reaction cuvette. The cuvette was placed in the Biocounter which was then set to give automatic injection of the luciferin-luciferase reagent into the cuvette. Light output over 10 seconds was measured. According to the instruction manual a reading of 800 RLU was equivalent to a bacterial count of 10^5 CFU/cm^3 in the original urine sample.

Results

Four hundred and twenty-two urines were tested, of which 83 showed a significant growth of bacteria ($\geq 10^5$ CFU/cm^3). Table 1 shows the results of both microscopy and bioluminescence on these 422 urines.

Table 1 *Analysis of 422 urines by strip counts, bioluminescence and microscopy*

Bacterial count (CFU/cm³)	*Strip culture*	*Bioluminescence (RLU)*		*Microscopy (WBC/mm³)*	
		≥800	*<800*	*≥200*	*<200*
$\geq 10^5$	83	75	8	49	34
$<10^5$	339	62	277	60	279

In terms of significant bacteriurias missed, bioluminescence, using a cut off point of 800 RLU (10%), was superior to urine microscopy as routinely performed in our laboratory (40%) (Table 2). Both techniques, however, showed a similar number of samples as positive, which on culture grew less than 10^5 CFU/cm^3 (18%). The eight significant bacteriurias missed by bioluminescence included four *Escherichia coli*, two *Pseudomonas aeruginosa*, one *Serratia sp* and one mixed growth containing both *E coli* and *Ps aeruginosa*. Of the 62 negative urines reported as positive by bioluminescence, 22 grew coagulase-negative staphylococci in pure culture, while 21 yielded no growth. Five of the latter contained large numbers of red cells and four large numbers of epithelial cells. Table 3 shows the bacterial taxa isolated from urines within given levels of bioluminescence readings.

Table 2 *Comparative performance of bioluminescence and microscopy as screening methods for urine*

	Bioluminescence	*Microscopy*
Agreement with strip culture	352/422 (84%)	328/422 (78%)
Positive cultures missed	8/83 (10%)	34/83 (40%)
Negative cultures reported as positive	62/339 (18%)	60/339 (18%)

Table 3 *Relation between bacterial taxa found in urine at bioluminescence magnitude*

Bacterial taxon found	*Culture negative*		*Culture positive*	
	<800RLU	*≥800RLU*	*<800RLU*	*≥800RLU*
Citrobacter	0	0	0	2
Enterobacter	0	0	0	3
Escherichia	18	16	5	48
Klebsiella	1	0	0	7
Proteus	4	3	3	12
Providencia	0	0	0	2
Pseudomonas	1	1	2	2
Serratia	0	0	1	0
Staphylococcus	16	22	0	12
Streptococcus	7	3	0	13
Non-fermentative	1	1	0	1
Fermentative	3	0	0	0
Coliform	8	6	0	0

The effectiveness of both screening techniques depends on the threshold value taken as being significant. For bioluminescence this was 800 RLU; for microscopy 200 WBC/mm^3. Figures 1 and 2 show the effect of varying these thresholds upon both the significant positive urines missed and the negative urines reported as positive.

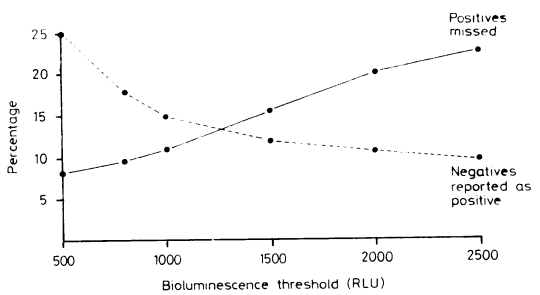

Fig. 1 *Effect of altering the level of bioluminescence taken as being equivalent to 10^5 CFU/cm^3.*

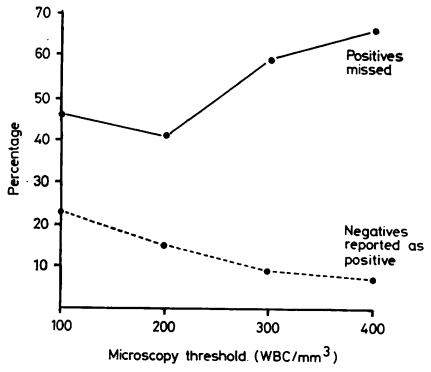

Fig. 2 *Effect of altering the level of the WBC count in urine taken as indicating potential infection.*

The reproducibility of the kit was tested on 12 samples in quadruplicate with mean bioluminescence readings of 242-51296 and was found to be poor particularly around the cut-off point (800 RLU) (Table 4).

Table 4 *Reproducibility of bioluminescence*

Urine	*Mean (RLU)*	*Standard deviation*	*CV %*
1	242	145	59·9
2	334	147	44·0
3	361	280	77·5
4	555	154	27·7
5	703	424	60·3
6	708	507	71·6
7	921	149	16·2
8	2224	969	43·5
9	2493	1337	53·6
10	3295	673	20·4
11	47 622	10 883	22·8
12	51 296	8502	16·6

Table 5 *Comparison of the working time, completion time and cost of processing 100 urine samples*

	Strip culture	*Microscopy*	*Bioluminescence*
Completion time (h)	18·0	4·0	3·5
Working time (h)	1·0	1·5	1·0
Cost of materials (£)	2·00	0·50	45·00

Table 5 shows the completion time, actual working time and the cost of processing 100 samples by strip culture, microscopy and bioluminescence. Although the last two were comparable in terms of time, the cost of bioluminescence was 90 times greater. The kit required a considerable amount of accurate pipetting and was less suitable for processing small numbers of urines as the 45-minute incubation stage was independent of the number of urines tested and the shelf-life of the reconstituted reagents was short. The Biocounter was very easy to use, but during the trial period developed two faults. The automatic injection system failed and the spring loaded piston used for ejecting the cuvettes stuck in the down position several times and was difficult to release.

Discussion

Bioluminescence was superior to microscopy of uncentrifuged urine at detecting urines with significant bacterial counts (90% accuracy as against 60%). Microscopy can be made more efficient at the expense of time and simplicity.[6 7] Failure of the screening test to detect these urines could result in a 24-hour delay in reporting the antibiotic sensitivity of the infecting strains. Bioluminescence was, however, not accurate enough to replace strip culture. The eight false-negatives obtained by bioluminescence included several different Gram-negative bacilli. None of these patients was on antibiotics.

Bioluminescence (62/399) and microscopy (60/399) gave similar numbers of false-positive results. Of the 62 urines in this category, 21 contained coagulase-negative staphylococci. In addition to MacConkey's medium, a non-selective medium (CLED) was used for strip cultures which should have supported the growth of coagulase-negative staphylococci, but it may be that bioluminescence is more effective in detecting these organisms. Bailey[8] has suggested that the normal level of significant bacterial counts in urine (10^5 CFU/cm^3) taken as being significant, may be too high for these staphylococci and that a count of $\geqslant 10^4$ CFU/cm^3 might be more appropriate. Eight of these 21 "false-positives" had counts between 10^4 and 10^5 CFU/cm^3.

Twenty-one of the bioluminescence false-positives showed no bacterial growth, but nine of these had high cell counts. The bacteriuria kit depends on the specific action of the mammalian and microbial ATP extracting reagents NRS and NRB. Failure to release ATP from host cells at the correct stage or incomplete action of the apyrase could result in false-positive results. Alternatively, the premature release of microbial ATP which could then be destroyed by apyrase might give false-negative results.

The threshold bioluminescence level used was 800 RLU. This was an arbitrary figure given in the instruction leaflet and assumed that different bacterial species in different phases of growth had a similar ATP content.

No rationale was given for the selection of the 800 RLU as the level of bioluminesencce equivalent

to 10^5 CFU/cm^3. Increasing the threshold level to 2500 RLU lowered the percentage of false-negative results from 25 to nine but also raised the percentage of positive cultures missed from 10 to 23.

The main criticisms of the luminescence technique are the low level of reproducibility, particularly near the cut-off point of 800 RLU, the large amount of manual involvement and the high cost. The first two problems could probably be overcome theoretically by the use of an automated method similar to that described by Johnston *et al.*[9] Unfortunately, none of the standard luminescence photometers currently available can be used with a continuous flow system and full automation is therefore not possible. This still leaves the cost of both the Biocounter (over £5000) and the reagents (45p per test), the latter being largely due to the luciferin-luciferase reagent. We therefore attempted to reduce the unit cost by using this reagent at a greater dilution than recommended by Lumac. Reliable results could not, however, be obtained with a five-fold dilution of luciferin-luciferase reagent.

The Lumac kit shows that bioluminescence can be used as a screening method for urine bacteriology. It is not, however, accurate enough to replace the strip count and it will not detect mixed microbial growths. Urine microscopy has other uses besides acting as a screening method for significant bacteriuria—for example, the detection of red cells, casts, and crystals; these functions bioluminescence cannot replace. Unless the cost per test can be reduced by a factor of 10, bioluminescence is not a realistic proposition for the detection of bacteriuria in the routine laboratory.

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