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Analyses of the FlashTrack DNA Probe and UTIscreen Bioluminescence Tests for Bacteriuria

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Five hundred urine specimens were selected at random and screened for bacteriuria by a DNA probe method, FlashTrack (Gen-Probe, San Diego, Calif.), and an automated bioluminescence method, UTIscreen (Los Alamos Diagnostics, Los Alamos, N.M.), and the results were compared with those of the semiquantitative plate culture method. The performance of each test versus culturing was evaluated at colony counts of $\geq 10^4$, $\geq 5 \times 10^4$, and $\geq 10^5$ CFU/ml. Since the interpretive breakpoint of each test was user selectable, the results were reported as receiver operator characteristic curves. Optimum interpretive breakpoints were determined for each test at each colony count by calculating a performance index that emphasized sensitivity over specificity in a 70:30 ratio. Although both tests had less-than-optimal sensitivities and specificities, the performance of FlashTrack was significantly better than that of UTIscreen at two of the three colony counts (10^4 and 10^5 CFU/ml); however, FlashTrack costs more and is a labor-intensive procedure. Neither method was evaluated for the detection of colony counts of $<10^4$ CFU/ml.

Urinary tract infections affect up to one-fifth of women and account for significant expense and morbidity, as well as increased mortality among hospitalized patients (7, 8, 15). For these reasons, urine specimens are among the most common specimens processed by microbiology laboratories. Since up to 80% of these may be interpreted as negative by commonly used colony count criteria, an accurate, cost-effective method for rapid urine screening would allow rapid reporting of negative results and more efficient treatment of patients and operation of laboratories.

Many urine screening tests that include microscopic, photometric, filtration, enzymatic, and bioluminescence techniques, among others, have been developed (9, 13, 14, 20, 21). While most of these methods have acceptable sensitivities, specificities, and predictive values at colony counts of 10^5 CFU/ml or greater, these performance parameters tend to be unacceptable at lower colony counts. Lower colony counts, such as 10^2 to 10^4 CFU/ml, however, may be a more accurate predictor of urinary tract infections, particularly in patients with a high prevalence of disease or those who are symptomatic or catheterized (10, 11, 16–19).

Recently, a chemiluminescent labelled DNA probe test for urine screening (FlashTrack; Gen-Probe, San Diego, Calif.) became available. In this study, we compared the FlashTrack DNA probe test and a recently automated bioluminescence method (UTIscreen; Los Alamos Diagnostics, Los Alamos, N.M.), with the standard semiquantitative plate count method as the reference method.

MATERIALS AND METHODS

Specimens. On each day of the study, approximately 25 midstream clean-catch and catheter urine specimens collected from both inpatients and outpatients and submitted to the microbiology laboratory at Bellevue Hospital were randomly selected, until a total of 500 urine specimens were examined. The specimens were either processed immediately or stored at 4°C for no longer than 12 h prior to being processed. After being tested, all specimens were stored at 4°C for 24 h for possible further testing to resolve discordant results.

Culturing. With a calibrated loop, 0.001 ml of urine was taken from each specimen and streaked onto both Trypticase soy agar–5% sheep blood and MacConkey agar plates (1). After incubation for 18 to 24 h at 35°C, colonies were counted to determine the total number of CFU per milliliter. All pure cultures, with the exception of diphtheroids, lactobacilli, and viridans streptococci, were considered to be probable pathogens. Mixed cultures were defined as any cultures with two or more colony types present.

UTIscreen. For UTIscreen, urine specimens were processed in accordance with the instructions of the manufacturer. Two negative controls, two reagent controls, and two positive standards were run with each batch of specimens. A 0.025-ml sample of well-mixed urine was added to a tube containing a somatic cell ATP-releasing agent. The tubes were incubated at room temperature for 15 min and placed in the carousel of a luminometer (model 633; Los Alamos Diagnostics). A bacterial ATP-releasing agent and luciferin-luciferase reagents were added automatically to the tubes, and the integrated light output was measured and displayed as a percentage of the average of two standards. A specimen was classified as positive or negative on the basis of an interpretive breakpoint selected by the user.

FlashTrack. Each of the 500 urine specimens was also tested with the FlashTrack urine screening test in accordance with the manufacturer's instructions. Each sample run included a positive control, two positive standards, and a negative standard. One milliliter of well-mixed urine was pipetted into a tube, which was centrifuged for 10 min at 1,800 × *g*. The supernatant was discarded, and the tubes were blotted on paper towels to remove the excess fluid. After 2 ml of wash solution was added to each tube, the tubes were again centrifuged at 1,800 × *g* for 10 min and the supernatant was discarded in a similar manner. To each tube 50 μl of lysis reagent was added, and the tubes were shaken and incubated for 15 min at 37°C. To each tube 50 μl of probe reagent was added, and the tubes were shaken and incubated

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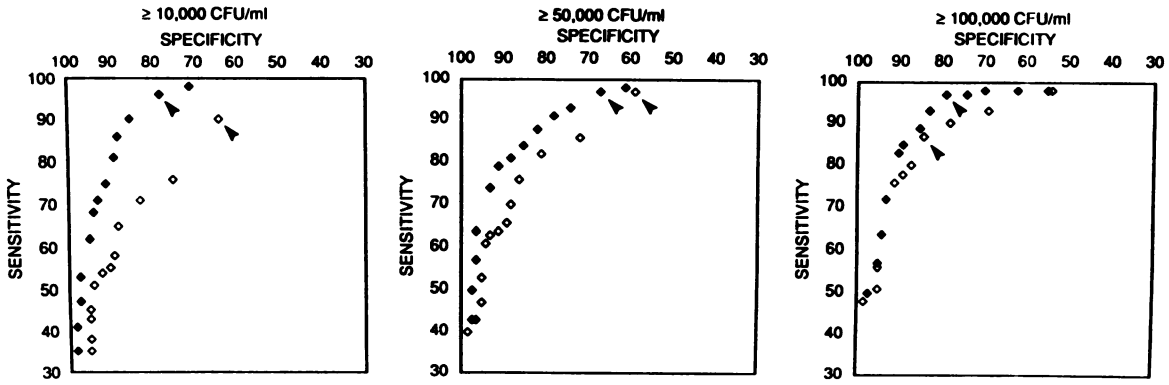

FIG. 1. Receiver operator characteristic curves for UTIscreen (◇) and FlashTrack (◆) at three different colony counts. The arrowheads indicate the optimum interpretive breakpoints based on a 70:30 weighting of sensitivity and specificity, respectively.

for 15 min at 60°C. Subsequently, 300 μl of selection reagent was added to each tube, and the tubes were vortexed three times for 3 to 4 s each time and incubated for 15 min at 60°C. Following the final incubation, the tubes were again vortexed three times for 3 to 4 s each time and allowed to cool for 5 min. Chemiluminescence was detected in a Leader-I luminometer (Gen-Probe). Results were calculated in gross relative light units and CFU per milliliter and printed automatically. A specimen was classified as positive or negative on the basis of an interpretive breakpoint selected by the user. The total processing time was approximately 2 to 2.5 h.

False-positives. All urine specimens that were evaluated as positive by either UTIscreen or FlashTrack and that were evaluated as negative by culturing were considered false-positives for the purposes of this study and were further analyzed. A Gram-stained smear of well-mixed uncentrifuged urine was examined and considered positive when an average of one or more bacteria per oil immersion field was seen (2), with a minimum of 10 fields per specimen being examined. For detection of the presence of fastidious organisms, 0.001 ml of well-mixed urine was streaked onto a chocolate agar plate with a calibrated loop; the plate was incubated in a candle jar for 48 h at 35°C, and then colony counting was performed. In addition, the original blood and MacConkey agar plates were reincubated for an additional 24 h at 35°C, and a second colony counting was performed. To assay for the presence of residual antimicrobial agents in the urine, we melted 20 ml of Trypticase soy agar, allowed it to cool to 55°C, and inoculated it with an aliquot of a nutrient broth culture of *Staphylococcus aureus* 209P (ATCC 6538P; American Type Culture Collection, Rockville, Md.) to a final concentration of approximately 10^5 CFU/ml. This suspension was poured into petri dishes (100 by 15 mm) and refrigerated. A 3-mm-diameter hole punch was used to cut wells into the agar of the inoculated assay dishes. These wells were filled with 20 μl of well-mixed urine, and the dishes were incubated for 18 to 24 h at 35°C. Since *S. aureus* 209P is used to assay for a broad range of antibiotics (3), any zone of inhibition around the wells likely indicated the presence of residual antibiotics in the urine.

Data analysis. Each of the 500 samples was classified as positive or negative for a variety of interpretive breakpoints. The results were compared with the results of the reference method, semiquantitative plate culturing, at $\geq 10^4$, $\geq 5 \times 10^4$, and $\geq 10^5$ CFU/ml to determine sensitivity, specificity, and positive and negative predictive values (5). Receiver operator characteristic curves were made for each test at each colony count to illustrate the relationship between sensitivity and specificity over the range of interpretive breakpoints (5). A performance index was determined for each interpretive breakpoint by use of a 70:30 weighted sum of sensitivity and specificity. The interpretive breakpoint for each colony count threshold was chosen to optimize the values of the performance index. In a sense, we chose the breakpoints to give the test its "best face." Differences in the performance indices of FlashTrack and UTIscreen were evaluated for statistical significance by calculation of large sample *t* ratios, which exceeded the 5% level for all values and the 2% level for most of the values. With respect to comparisons of the two test methods, there was no need to consider sampling variability about the population, except for quantities involving predictive value, which depends on prevalence. However, this study was mainly concerned with a comparison of the two test methods against a "gold standard" whose characteristics have been well studied.

RESULTS

Of the 500 urine samples tested, 148 had $\geq 10^5$ CFU/ml, 182 had $\geq 5 \times 10^4$ CFU/ml, and 234 had $\geq 10^4$ CFU/ml, as determined by culturing. Figure 1 shows the receiver operator characteristic curves for each test at each colony count. The interpretive breakpoints were varied from 1 to 40% of the integrated light output for UTIscreen and from 10,000 to 800,000 CFU/ml for FlashTrack. As expected, the lowest interpretive breakpoints gave the highest sensitivity and the lowest specificity, whereas the highest breakpoints gave the lowest sensitivity and the highest specificity. The arrowheads indicate the interpretive breakpoints that maximized the performance indices. These were 1, 1, and 4% of the integrated light output of the positive standards for UTI screen and 25,000, 25,000, and 100,000 CFU/ml for FlashTrack at $\geq 10^4$, $\geq 5 \times 10^4$, and $\geq 10^5$ CFU/ml, respectively.

Table 1 lists the performance parameters of the two tests at these breakpoints. The arithmetic difference between the performance indices of the two test methods had a large sample *t* ratio of 5.4 at $\geq 10^4$, 1.2 at $\geq 5 \times 10^4$, and 2.5 at $\geq 10^5$ CFU/ml, indicating a significantly better performance for FlashTrack at two of the three colony counts ($\geq 10^4$ and $\geq 10^5$). Although the difference was not statistically significant at $\geq 5 \times 10^4$ CFU/ml, the uniformity of the results offers ample evidence of the superiority of FlashTrack over UTI screen.

Of the 148 cultures with $\geq 10^5$ CFU/ml, 83 were pure

TABLE 1. Performance parameters for UTIscreen and FLASHTRACK compared with those of culturing at three colony counts

Test	CFU/ml	No. of specimens that were				%				Performance index
		True-positives	True-negatives	False-positives	False-negatives	Sensitivity	Specificity	Predictive value		
								Positive	Negative	
UTIscreen	10^4	210	170	96	24	90	64	69	88	82
	5×10^4	176	187	131	6	97	59	58	97	85
	10^5	129	296	56	19	87	84	70	94	86
FlashTrack	10^4	224	208	58	10	96	78	80	95	91
	5×10^4	177	213	105	5	97	67	63	98	88
	10^5	143	276	76	5	97	79	66	98	91

cultures of probable pathogens and were identified as *Escherichia coli* (n = 31), *Enterococcus* spp. (n = 10), *Candida* spp. (n = 9), *Klebsiella* spp. (n = 5), *Proteus* spp. (n = 4), coagulase-negative staphylococci (n = 4), *S. aureus* (n = 4), beta-hemolytic streptococci (n = 4), *Acinetobacter* spp. (n = 4), *Citrobacter* spp. (n = 3), *Pseudomonas* spp. (n = 3), and *Providencia* spp. (n = 2). UTIscreen detected 70 (84%) and FlashTrack detected 81 (98%) of the 83 probable pathogens, percentages which were similar to their sensitivities for all isolates at $\geq 10^5$ CFU/ml (87 and 97%, respectively). In addition, 93 of 182 urine samples with $\geq 5 \times 10^4$ CFU/ml and 116 of 234 with $\geq 10^4$ CFU/ml were pure cultures of probable pathogens. Similarly, the sensitivities of both tests for probable pathogens at these colony counts were unchanged, except for an improvement from 90 to 97% for UTIscreen at $\geq 10^4$ CFU/ml.

All false-positive specimens for both tests were further examined as described above. At $\geq 10^5$ CFU/ml, 55 total false-positive specimens were observed with UTIscreen. Thirty-two of these (58%) were found to contain significant numbers of bacteria by Gram staining (n = 27), a colony count of $\geq 10^5$ CFU/ml after reincubation (n = 8), a colony count of $\geq 10^5$ CFU/ml after plating on chocolate agar (n = 15), or some combination of these methods. Similarly, 75 urine specimens were FlashTrack positive and culture negative at $\geq 10^5$ CFU/ml. Sixty-two of these 75 (83%) were found positive by at least one of the following methods: Gram staining (n = 52), reincubation (n = 17), or growth on chocolate agar (n = 32). Significant numbers of false-positive specimens for both tests at $\geq 10^4$ and $\geq 5 \times 10^4$ CFU/ml were also accounted for by these three supplementary procedures. Of the organisms showing growth on chocolate agar, one was *Haemophilus influenzae* and the remainder were either diphtheroids or lactobacilli. Of the 64 urine specimens tested for antimicrobial agents, 33 (52%) showed well-defined zones of inhibition around the wells. Twenty-six of these urine specimens were not found positive by any of the other three supplementary procedures, thus providing an additional explanation for false-positive specimens.

DISCUSSION

We evaluated the FlashTrack DNA probe method for urine screening and reported our results as receiver operator characteristic curves. This was done since both of the tests examined (FlashTrack and UTIscreen) allow the user to determine the cutoff for a positive test on the basis of the desired sensitivity and specificity for the user's laboratory. To compare the performance parameters of the two tests, we needed to choose an optimum interpretive breakpoint for each test at each colony count. This was done by determining for each point on the curve a performance index that was somewhat arbitrarily defined as 70% of the sensitivity plus 30% of the specificity. Sensitivity was weighted more heavily, since these are screening tests and, in our opinion, the consequences of a false-negative result are greater than those of a false-positive result. Small changes in the weighting of sensitivity versus specificity did not alter the optimum interpretive breakpoints.

As expected, the receiver operator characteristic curves for both tests indicated good performance at $\geq 10^5$ CFU/ml, with FlashTrack showing only a slightly better sensitivity. As the colony count was decreased, however, to $\geq 5 \times 10^4$ CFU/ml and finally to $\geq 10^4$ CFU/ml, the curves become progressively more divergent, with FlashTrack maintaining its position but UTIscreen showing a marked drop in performance, especially in sensitivity. Similarly, when the maximum performance indices were compared, FlashTrack performed significantly better than UTIscreen at two of the three colony counts.

Although FlashTrack performed better than UTIscreen, it has two major drawbacks, high cost and a very complex protocol. The FlashTrack procedure has two centrifugation steps and three incubation steps and requires the manual addition of reagents for a total of 2 to 2.5 h before results are obtained. The test is therefore more expensive because of increased labor as well as the high cost of reagents. Our results with UTIscreen were comparable to those in previously published reports of the bioluminescence method (4, 6, 12, 20). While not performing as well as FlashTrack, UTI screen is fully automated, requiring only a single pipetting step after calibration of the instrument. Reagents are added and results are printed automatically for up to 23 specimens at a time.

We found that large numbers of urine specimens found false-positive by either test contained significant numbers of bacteria, as determined by Gram staining, extended incubation, and/or growth on chocolate agar. This result points out the limitations of the semiquantitative plate culture method for detecting fastidious bacteria and indicates that the "true" sensitivities of these tests may in fact be higher than what is shown in Table 1. Admittedly, the significance of detecting these bacteria in urine is questionable, since the vast majority most likely represent contaminants. Grossly bloody specimens showing no growth in cultures consistently gave a false-positive UTIscreen result. This limitation of bioluminescence technology is a well-recognized one, since erythrocytes contain ATP; however, it accounted for a small number of false-positives. Both tests showed similar proportions of false-negatives for all isolates and probable pathogens, except for UTIscreen at $\geq 10^4$ CFU/ml. This improved sensitivity for probable pathogens at low concen-

trations may be a result of contaminants experiencing an initial "lag phase" of growth during which they do not have sufficient ATP to produce a positive result.

Although the performance of the FlashTrack DNA probe test compares very favorably with that of the more established bioluminescence method, we feel that both tests lack sufficient sensitivity and specificity to reliably detect low concentrations of bacteria, which may be significant in symptomatic and catheterized patients. In addition, the FlashTrack procedure is far too labor-intensive to be used as a practical screening test. While DNA probe technology is well suited to identifying organisms that are difficult to culture, its applicability to screening urine specimens at this time is limited by the expense and labor required to perform the procedure. On the other hand, while the UTIscreen bioluminescence test is easy to perform, in our analysis it shows a marked loss of specificity in order to obtain an acceptable level of sensitivity at lower colony counts. Thus, this study further emphasizes the need for a highly sensitive and specific urine screening test that is both economical and technically easy to perform.

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