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Clinical Laboratory Evaluation of a Urine Screening Device

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A study was conducted to compare the Bac-T-Screen Bacterial Detection Device for Urines (BDD; Marion Laboratories, Kansas City, Mo.) with urine Gram stain as a screen for bacteriuria. We analyzed 631 urine samples with the BDD and compared the results to urine Gram stains and quantitative cultures. A total of 90 (14%) specimens could not be analyzed with the BDD due to interfering pigments (67 specimens) or clogging of the filter (23 specimens). Of the 541 specimens that were analyzed, the BDD correctly identified 67 (88.2%) of the 76 specimens with $\geq 10^5$ CFU/ml but only 294 (63.2%) of the 465 specimens with $<10^5$ CFU/ml. The majority of the false negative specimens had either gram-positive organisms or yeasts. The predictive value of a negative BDD reading was 97.0%. The urine Gram stain correctly identified 92.1% of all positive cultures and 77.8% of all negative cultures. The predictive value of a negative urine Gram stain was 98.4%. In summary, the BDD compares favorably with the urine Gram stain as a screen for bacteriologically negative urine specimens.

Urine cultures constitute a significant proportion of the specimens processed in most clinical microbiology laboratories. In an effort to reduce the amount of time required to process these specimens and to produce more timely results for patient management, a number of tests have been developed to screen specimens for the presence of large numbers of organisms.

These methods include both growth-dependent and growth-independent techniques. Techniques dependent on microbial growth include the early inspection of broth cultures and agar plates inoculated with urine (16) and measurement of bacterial growth by electrical impedance (3), microcalorimetry (2), and photometry (18). Many of these techniques can be automated, although the cost of the instruments and consumable supplies can be high. Growth-independent techniques include biochemical tests such as the nitrite or glucose tests (7, 15), leukocyte esterase determination (17), detection of endotoxin (10), measurement of bacterial ATP (5), quantitation of bacteria with a particle counter (20), and microscopic examination of urine for the presence of bacteria (10, 12, 14) or pyuria (6, 19, 22) or both. The results of these techniques can be obtained much faster in comparison with the growth-dependent tests. However, the growth-independent tests are not used extensively in clinical labs because they are either insensitive or technically demanding (1, 5, 10, 19).

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Recently, Wallis and co-workers (23) reported an innovative method for detecting bacteriuria. They used a semiautomated instrument (Bac-T-Screen Bacterial Detection Device [BDD]; Marion Laboratories, Kansas City, Mo.) to concentrate organisms in the urine specimen onto a filter and then to stain the trapped organisms. The intensity of the stain retained in the filter would theoretically be proportional to the number of organisms in the urine specimen. This procedure was reported to be technically simple, rapid (total testing time is less than 1 min), and accurate in detecting specimens with $\geq 10^5$ organisms per ml of urine. In the study reported here, we attempted to confirm and extend the work of Wallis and co-workers by comparing results obtained with the BDD with quantitative urine cultures and Gram stains of uncentrifuged urine.

MATERIALS AND METHODS

Specimens and quantitative cultures. A total of 631 urine specimens, collected during a 9-week period, were processed in the Barnes Hospital Clinical Microbiology Laboratory. Midstream (81%) and catheterized (19%) urine specimens were collected in sterile containers and were stored in the laboratory at 4°C until processed. All specimens were cultured within 2 h of receipt. A 0.01-ml calibrated platinum loop was used to inoculate the urine specimens onto tryptic soy agar with 5% defibrinated sheep blood and MacConkey agar plates. Colony counts were determined after incubation at 35°C for 24 and 48 h, and bacterial and fungal isolates were identified by conventional procedures (13). Growth was recorded as $<10^4$, 10^4 to $<10^5$,

or $\geq 10^5$ CFU/ml. The criterion for significant bacteriuria used in this study was a colony count of $\geq 10^5$ CFU/ml as defined by Kass for asymptomatic patients (11). After the initial processing, the specimens were refrigerated at 4°C until the screening tests were performed.

Urine screening with the BDD. All specimens were screened by the BDD in the following manner. A filter was placed into the first barrel of the instrument, and a 1-ml portion of a well-mixed specimen of urine was poured into the barrel. Upon activation of the instrument, an acid diluent was added to the urine to lyse any nonbacterial cells. The urine-diluent mixture was then automatically removed by vacuum filtration, thus trapping organisms on the filter. Immediately after filtration of the urine, safranin was automatically dispensed into the barrel to stain the trapped organisms. The dye was then removed by vacuum and followed by the first decolorizing solution. After the decolorizing solution was removed, the filter was manually transferred to a second barrel where additional decolorizing solution was dispensed. After the final decolorization-filtration step, the filter was removed and examined for retention of the stain. The color intensity of the filter was compared with a color chart and ranked as negative (no color), +/− (very slight pink color), or 1+, 2+, 3+, or 4+ for increasing intensity of the pink-red color (23). For the purpose of this study, staining intensity of +/− or greater was considered positive. Specimens that clogged the filter or produced staining due to urinary pigments were excluded from further analysis. All filters were read by at least two independent observers at different times. No difference in staining intensity was observed between wet and dry filters, and the stained filters did not appear to fade when stored at room temperature for as long as 3 weeks. However, the staining property of the filters was not stable beyond this time interval.

Urine screening by Gram stain of uncentrifuged urine. A portion of each urine specimen was mixed, Gram stained, and then examined under oil immersion for 1 to 2 min. Results of the microscopic examination were recorded as no organisms seen, <1 organism per high-power field (HPF), and ≥1 organism per HPF. All specimens with ≥1 organism per HPF were considered positive by this technique. These results were compared with quantitative cultures and the BDD results.

Statistical calculations. Statistical analysis was performed by chi-square testing with Yates' correction (4). The sensitivity, specificity, and predictive value of a positive or negative test were calculated for the two screening tests as follows (8):

$$\text{Sensitivity (\%)} = \frac{\text{True positive}}{\text{True positive + false negative}} \times 100$$

$$\text{Specificity (\%)} = \frac{\text{True negative}}{\text{True negative + false positive}} \times 100$$

$$\text{Predictive value of positive (\%)} = \frac{\text{Correct positive by screen}}{\text{Total positive by screen}} \times 100$$

$$\text{Predictive value of negative (\%)} = \frac{\text{Correct negative by screen}}{\text{Total negative by screen}} \times 100$$

The results of the quantitative cultures were the basis for establishing the true positive and true negative test results.

RESULTS

Of the 631 urine specimens processed during the study, 90 (14%) could not be analyzed with the BDD due to interfering pigments (67 specimens) or clogging of the filter (23 specimens). The remaining 541 specimens were processed as described above. A total of 76 specimens (14.0%) contained $\geq 10^5$ CFU/ml, 102 (18.9%) contained between 10^4 and 10^5 CFU/ml, and 363 (67.1%) contained $<10^4$ CFU/ml.

The results of screening the urine specimens with the BDD and Gram stain are summarized in Tables 1 and 2, respectively. The BDD correctly identified 67 (88.2%) of the 76 specimens with $\geq 10^5$ CFU/ml but only 294 (63.2%) of the 465 specimens with $<10^5$ CFU/ml. In comparison, if ≥1 organism per HPF were considered positive, then the Gram stain correctly identified 70 (92.1%; $P > 0.1$) of the specimens with $\geq 10^5$ CFU/ml and 362 (77.9%; $P < 0.001$) of the specimens with fewer organisms. Both screening procedures were more sensitive in detecting significant numbers of gram-negative bacteria compared with gram-positive organisms (Table 3). Of the 53 gram-negative bacteria present at $\geq 10^5$ CFU/ml, 94.3% were detected with the

TABLE 1. Screening of urine specimens with the BDD

Quantitative culture results (CFU/ml)	Total no. of specimens with the following characteristics							
	BDD staining intensity						Pigmented	Clogged
	Negative	+/−	1+	2+	3+	4+		
$\geq 10^5$	9	5	7	15	25	15	3	9
10^4–$<10^5$	30	39	16	9	4	4	8	6
$<10^4$	264	73	16	7	2	1	56	8

TABLE 2. Screening of urine specimens with Gram stain

Quantitative culture result (CFU/ml)	Total no. of specimens with:		
	No organisms seen	<1 organism/ HPF	≥1 organism/ HPF
$\geq 10^5$	5	1	70
10^4–$<10^5$	32	9	61
$<10^4$	298	23	42

BDD and 98.1% by Gram stain. In contrast with this, only 77.8% of the 27 gram-positive organisms were detected by the BDD and 81.5% by Gram stain ($P > 0.1$). Table 4 is an analysis of the BDD and Gram stain results obtained in this study for all urine specimens with $\geq 10^5$/ml, regardless of organism type, in comparison with similar data previously reported for three automated instruments (18). The predictive value of a positive test (i.e., the proportion of positive test results that were true positives) was unacceptable for both the BDD and Gram stain (28.0 and 40.3%, respectively [$P < 0.05$]). However, this was also a problem with the automated instruments. In contrast with this, the predictive value of a negative test (i.e., the proportion of negative test results that were true negatives) was very good for both the BDD and Gram stain (97.0 and 98.4%, respectively [$P > 0.1$]), as well as with the three instruments.

DISCUSSION

Theoretically, the most rapid screening tests for significant bacteriuria are those that are independent of microbial growth. Thus, several biochemical tests (e.g., nitrate reduction, glucose utilization, leukocyte esterase activity, and endotoxin assay) have been devised that can provide an answer in minutes rather than in hours. However, these tests are either too insensitive or too specific (e.g., they only detect gram-negative organisms producing endotoxin) to be useful. Although the Gram stain is a growth-independent test that is rapid and inexpensive, the reported sensitivity of the test varies widely due to the technical difficulty in interpreting the stain (6, 10, 14, 19).

Attempts to develop or adapt instrumentation for use in urine screening have been moderately successful, with the most popular instruments utilizing photometry to monitor microbial growth. However, these instruments and their consumable supplies are relatively expensive. Additionally, incubation of the urine specimen for as long as 5 to 13 h may be required before the test result is available. Thus, the photometric instruments can provide standardized results that are not possible with the Gram stain. However, their significant expense and slow testing time make the photometric instruments unattractive for screening urine specimens. The BDD was developed in an attempt to provide a screening test for significant bacteriuria that has the speed of the Gram stain and the objectivity of the photometric instruments. The BDD is

TABLE 3. Summary of the detection of organisms by the BDD and Gram stain

Organisms	No. of isolates[a]	No. detected by:	
		BDD	Gram stain
Gram negative			
Escherichia coli	28	28	27
Klebsiella spp.	8	8	8
Proteus spp.	4	3	4
Serratia sp.	1	1	1
Enterobacter spp.	2	2	2
Citrobacter spp.	4	4	4
Providencia sp.	1	1	1
Pseudomonas spp.	3	2	3
Acinetobacter sp.	1	1	1
Gardnerella sp.	1	0	1
Gram positive			
Staphylococcus epidermidis	6	4	5
Staphylococcus aureus	1	1	1
Streptococcus spp.			
Group D	6	5	4
Other	5	2	3
Lactobacillus spp.	3	3	3
Corynebacterium spp.	4	4	4
Yeasts	2	2	2

[a] A total of 80 organisms ($\geq 10^5$ CFU/ml) were isolated in 76 urine specimens.

TABLE 4. Comparative analysis of five screening tests[a]

Screening test	Sensitivity (%)	Specificity (%)	Predictive value (%) of:	
			Positive test	Negative test
BDD	88.2	63.2	28.0	97.0
Gram stain	92.1	77.8	40.3	98.4
Autobac	85.3	84.4	47.0	92.2
MS-2	74.9	87.1	48.6	95.3
AMS	82.8	72.4	32.9	96.3

[a] Values of BDD and Gram stain were calculated from data for all urine samples with $\geq 10^5$ CFU/ml presented in this study; sensitivity and specificity values for Autobac (General Diagnostics, Warner-Lambert Co., Morris Plains, N.J.), MS-2 (Abbott Laboratories, Diagnostics Div., Irving, Tex.), and AMS for all urine samples with $>10^5$ CFU/ml, regardless of organism type, from Pezzlo et al. (18); predictive values for the three instruments were calculated by using the incidence of significant bacteriuria reported in this study (e.g., 14.0%).

technically very simple with only three steps required for processing a specimen: (i) concentration of organisms onto a filter, (ii) selective staining of the trapped organisms, and (iii) decolorization of all stains not retained by the organisms. Thus, the BDD should be equivalent to the Gram stain in detecting the presence or absence of significant bacteriuria, because both methods are based on the ability of bacteria to take up stain. In addition, the BDD potentially is a more objective test than the urine Gram stain. The initial report by Wallis and co-workers demonstrated that the BDD was rapid (less than 1 min per test) and simple to interpret, with very little technical expertise required to distinguish a positive from a negative test (23). By using the criteria of $\geq 10^5$ CFU/ml of urine as significant bacteriuria, the BDD was reported to have a sensitivity of 95% and a specificity of 98%.

In our experience, the BDD was very simple to operate and to interpret. There was no significant variation in the readings of either wet or dry filters between three observers. In addition, the test was very rapid averaging approximately 1 min per specimen. In contrast with the report of Wallis and co-workers, we found the overall sensitivity (88.2%) and specificity (63.%) to be somewhat low (Table 4). The reason for this discrepancy is unclear. Randomly collected urine specimens were processed in both studies, and the prototype instrument that they used was essentially the same as our instrument. In their study, Wallis et al. considered +/− BDD readings to be negative (23), whereas the manufacturer now interprets these as positive. If these were classified as negative in our study, the sensitivity of the BDD test would decrease from 88.2 to 81.6%, and the specificity would increase from 63.2 to 87.3%. These results are still different from those of Wallis et al. (23). The predictive value of a negative test (97.0%), however, was good, and the results for sensitivity and predictive value of a negative test obtained with the BDD in this study were not statistically different from those obtained with the urine Gram stain performed on the same samples (Table 4). Furthermore, the sensitivity and predictive values of the BDD were comparable to those reported for photometric instruments (18). Both the specificity and the predictive value of a positive test were significantly lower for the BDD than for the Gram stain ($P < 0.05$).

The BDD is potentially useful in several situations. Highly automated instruments, such as the AutoMicrobic system (AMS; Vitek Systems, Inc., Hazelwood, Mo.), are relatively slow and cost inefficient in processing negative urine samples (9). Thus, elimination of most of the negative urine samples by screening with the BDD would allow these instruments to process predominantly positive urine samples and function more efficiently. Another potential role for the BDD would be in screening urine specimens in an outpatient clinic or a physician's office. Because the vast majority of outpatient urinary tract infections are caused by gram-negative organisms (21), the superior ability of the BDD to detect significant gram-negative bacteriuria could be utilized. The feasibility of using the BDD in these two situations is in part dependent on the cost of the screening test. For example, if using the BDD to eliminate negative cultures is more expensive than processing all specimens with the AMS instrument, then a major potential value of the BDD would not be realized. Figure 1 is a summary of our analysis of the cost effectiveness of the BDD. The two major variables affecting the cost of a screening test for negative specimens is the cost for each individual test and the proportion of specimens that are negative. The cost for each test has not been established by the manufacturer. Therefore, we have plotted the curves for filters costing \$0.10, \$0.50, \$1.00, and \$1.50. The cost of technologists' time is not included because these estimates will vary widely for each laboratory and do not represent true savings unless the technical position is eliminated by the new test. If one assumes that approximately 85% of the specimens are negative (our experience in this study) and the cost of supplies for the AMS is \$2.93 (18), then the BDD is cost effective at all four estimated filter prices. However, if the proportion of negative specimens decreases, then the relative cost of detecting a negative urine sample progressively increases. Thus, if fewer than 50% of the urine specimens are negative, then the BDD is not cost effective at \$1.50 per filter. If the cost of supplies for performing a culture is \$0.67 (18), then the BDD is only cost effective if the filter costs \$0.50 or less. Obviously, factors other than cost per test must be considered in evaluating a new test. However, if the test significantly increases the cost of processing the culture, many laboratories will not be able to afford the new procedure.

In summary, the BDD compares favorably in terms of sensitivity and predictive value of a negative test with the urine Gram stain as a urine screening device. The advantages of the BDD over the Gram stain are speed and elimination of observer variability. The excellent predictive value of a negative test makes it useful for eliminating the negative urine specimens submitted to the microbiology laboratory. The rather low specificity makes quantitative culture of all positive specimens essential. All specimens producing interfering pigments or clogging of the filter should also be cultured quantitatively. In addition, if one suspects clinically significant

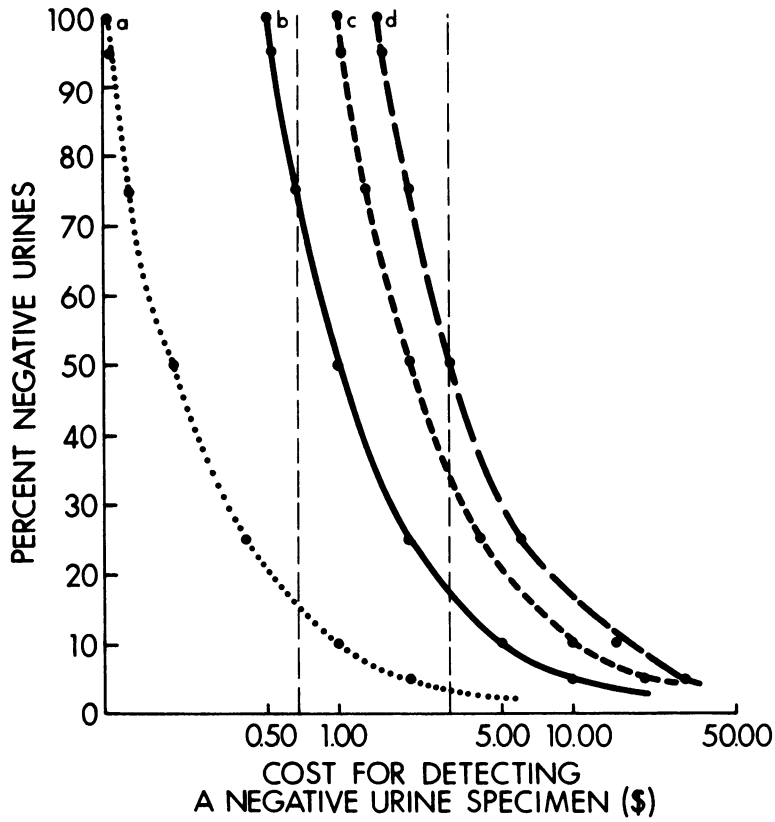

FIG. 1. Cost analysis of BDD. Plotted curves represent cost per BDD filter at $0.10 (a), $0.50 (b), $1.00 (c), and $1.50 (d). Data points representing the cost for detecting a negative urine specimen were derived by dividing the cost per BDD filter by the percent of negative urine. Horizontal lines are the previously determined cost (18) of supplies for a culture ($0.67) and an AMS test ($2.93).

bacteriuria at a level of $<10^5$ CFU/ml, then the specimen should be cultured quantitatively because the BDD and all other screening devices are relatively insensitive at this level of bacteriuria. Recent studies by Stamm and co-workers (22) have demonstrated that disease in symptomatic women may be caused by as few as 10^1 to 10^2 organisms per ml of urine. Therefore, all screening tests based on detecting $\geq 10^5$ organisms per ml may exclude a significant proportion of infected patients. Thus, the BDD can accurately identify specimens with $<10^5$ organisms per ml, a level of bacteriuria considered insignificant in asymptomatic patients (11). However, the clinical significance of this function for the general population remains to be determined.

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