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Evaluation of a Two-Minute Test for Urine Screening

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A study was conducted to evaluate the ability of a urine filtration system (Bac-T-Screen, Marion Laboratories, Inc., Kansas City, Mo.) to detect negative urine cultures within 2 min. A total of 1,000 urine specimens were tested with the Bac-T-Screen and compared with a standard semiquantitative culture plate method and the Autobac system (General Diagnostics, Warner-Lambert Co., Morris Plains, N.J.). Of the 1,000 clean voided urine specimens tested, 246 specimens had colony counts $\geq 10^5$ CFU/ml by the culture plate method. Of these, the Bac-T-Screen detected 65.4% (161 of 246), and the Autobac detected 63.0% (155 of 246). When pure cultures of diphtheroids, lactobacilli, and viridans streptococci other than group D and cultures containing multiple organisms were considered to be contaminants and, therefore, were excluded, there were 106 pure cultures of probable pathogens of which the Bac-T-Screen detected 76.4% (81 of 106) and the Autobac detected 90.6% (96 of 106). Some 133 specimens were uninterpretable with the Bac-T-Screen because 36 clogged the filter and 97 left a residual pigment on the filter. A majority of those clogging the filter (69.4%) had positive plate counts, whereas the majority of the pigmented urines had negative plate counts. Of those urine specimens tested, 754 were negative by the culture plate method. The false-positive rates for Bac-T-Screen and Autobac were 16.2 and 5.8%, respectively. As a urine screen, the Bac-T-Screen has a negative predictive value comparable to the Autobac system and has the advantage of being a 2-min test.

Many rapid methods for screening urine specimens for the presence or absence of bacteriuria have been described (2, 6, 7, 10, 12, 13, 15). These include microscopic, chemical, and automated methods, most of which require from 1 to 13 h before final results are available. The purpose of rapid screening is to provide information for physicians in a timely manner. A simple rapid screening method which provides immediate results, if effective, could increase laboratory efficiency, decrease laboratory costs, and also allow physicians to initiate prompt therapy if needed. In addition, a rapid method may encourage physicians to screen more of the asymptomatic population, thereby quickly detecting infections in patients, such as pregnant women and the elderly, who might be at risk for developing complications.

Wallis et al. recently described a bacteriuria detection device which provides results within 2 min (15). The Bac-T-Screen (Marion Laboratories, Inc., Kansas City, Mo.) is a colorimetric filtration system based on the presence of bacteria in urine rather than on their growth. The purpose of this investigation was to evaluate the ability of this system to detect negative urine specimens accurately within 2 min and to compare it with a standard semiquantitative culture plate method and the Autobac system (General Diagnostics, Warner-Lambert Co., Morris Plains. N.J.).

MATERIALS AND METHODS

Specimens. A total of 1,000 clean-voided urine specimens from both inpatients and outpatients submitted to the Medical Microbiology Laboratory at the University of California Irvine Medical Center, Orange, Calif., were included in the study. Patients receiving antimicrobial therapy were not excluded. Upon collection, urine was placed in a sterile tube and refrigerated (4°C) before processing. Specimens were tested within 4 h of collection.

Semiquantitative culture. A semiquantitative plate count as described by Barry et al. (1) was used as the reference method. By using a calibrated platinum loop, 0.001 ml of a well-mixed urine specimen was inoculated onto a 5% sheep blood agar plate and a biplate consisting of MacConkey agar and polymyxin B-nalidixic acid blood agar (CalLabs, North Hollywood, Calif.). Cultures were incubated at 35°C aerobically overnight and examined for the number and types of organisms present.

A positive culture was defined as a urine specimen which yielded $\geq 10^5$ CFU/ml (10). This category was further divided into significant positives and contaminants. Significant positives were defined as urine

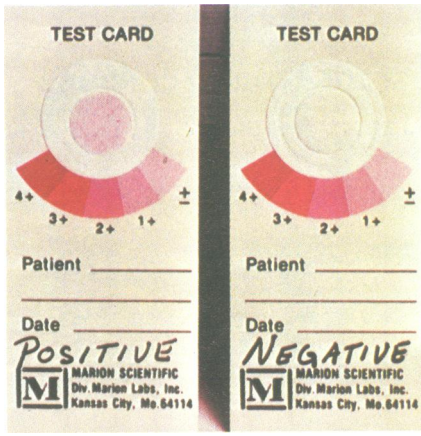

FIG. 1. Filter cards from positive and negative urine specimens.

specimens with a single potential pathogen, and contaminants were mixed cultures or those containing a pure culture of diphtheroids, lactobacilli, or viridans streptococci other than group D. Significant isolates were identified by standard biochemical procedures (11).

All urine cultures were divided into three categories based on the CFU per milliliter obtained by the semiquantitative plate culture method. Group 1 included all urine specimens with colony counts of $\geq 10^5$ CFU/ml; group 2 included those with colony counts from $\geq 10^4$ to $<10^5$ CFU/ml; and group 3 included specimens with colony counts of $<10^4$ CFU/ml.

Autobac. Urine specimens were tested by the Autobac urine screen according to manufacturer's instructions, as previously described (3–6, 9, 13). In this study, the only modification was that readings were taken at 1 and 4 h.

Bac-T-Screen. Urine specimens were processed with the Bac-T-Screen according to manufacturer's instructions and as previously described (15). One ml of well-mixed urine and 3 ml of urine diluent (14.5% acetic acid), followed by 3 ml of safranin O dye and a final rinse with 3 ml of 2.4% acetic acid decolorizer, were filtered through a piece of filter paper that was attached to a card (Fig. 1). The color intensity remaining was coded (± to 4+) using a color guide provided by the manufacturer. A positive test was one which gave a pink residual on the filter with an intensity of ≥1+.

Urinalysis. All urine specimens were tested for pH, protein, glucose, ketones, bilirubin, blood, and nitrite by using Ames Multistix (Miles Laboratories, Inc., Elkhart, Ind.). A portion of the urine was centrifuged (10 min, 1,000 × *g*), and the pellet was examined for crystals, casts, leukocytes (WBCs), and erythrocytes.

Predictive value. Predictive values were calculated by the method of Ransohoff and Feinstein (14). The sensitivity, specificity, predictive value positive, and predictive value negative, were calculated as follows: Sensitivity = TP/(TP + FN); specificity = TN/(TN + FP); predictive value positive = TP/(TP + FP); and predictive value negative = TN/(TN + FN), where TP is true positive, TN is true negative, FP is false-positive, and FN is false-negative.

RESULTS

Specimens used. A total of 1,000 clean-voided urine specimens were evaluated (Table 1). Of these, 246 were positive, with colony counts $\geq 10^5$ CFU/ml by the standard semiquantitative culture plate reference method (group 1). Group 2 included 118 urine cultures (11.8%) with colony counts between $\geq 10^4$ and $<10^5$ CFU/ml. The remaining 636 had colony counts of $<10^4$ CFU/ml and were placed in group 3. Of these, 543 showed no microbial growth.

Group 1. (i) Bac-T-Screen. Of the 246 specimens with colony counts of $\geq 10^5$ CFU/ml, 65.4% (161 of 246) were detected by the Bac-T-Screen. An additional 13.8% of the specimens (34 of 246) were uninterpretable by the Bac-T-Screen and therefore were not included in the 65.4% detection rate. Of these, 73.5% (25 of 34) clogged the filter and aborted the test, and 26.5% (9 of 34) left a residual orange-to-brown pigment on the filter card, interfering with the interpretation.

Within group 1 43.1% of the specimens (106 of 246) had pure cultures of probable pathogens. Of these, 76.4% (81 of 106) gave a positive reaction on the Bac-T-Screen filter card (Table 1). In this group, 20 specimens were uninterpretable since 18 of 106 (17.0%) clogged the filter and 2 of 106 (1.9%) left a residual pigment. Of the specimens

TABLE 1. Distribution of urine specimens

Group	No. of specimens	Concn (CFU/ml)	No. (%) of cultures			
			Autobac positive	Bac-T-Screen		
				Positive	Cloggers	Pigmenters
1						
All specimens	246	$\geq 10^5$	155 (63.0)	161 (65.4)	25 (10.2)	9 (3.7)
Pure pathogens	106	$\geq 10^5$	96 (90.5)	81 (76.4)	18 (17.0)	2 (1.9)
Contaminants	140	$\geq 10^5$	59 (42.1)	80 (57.1)	7 (5.0)	7 (5.0)
2	118	$\geq 10^4 < 10^5$	27 (22.9)	23 (19.5)	2 (1.7)	11 (9.3)
3	636	$<10^4$	17 (2.7)	88 (13.8)	9 (1.4)	77 (12.1)

clogging the filter, 16 grew *Escherichia coli*, and 2 grew *Klebsiella pneumoniae*. All contained a large number of WBCs. The two pigmented urines represented one isolate each of *Streptococcus agalactiae* and *E. coli*.

Of the remaining interpretable specimens, the Bac-T-Screen was unable to detect 4.7% of the isolates (5 of 106). These represented two isolates of enterococci and one isolate each of *E. coli*, *K. pneumoniae*, and *Staphylococcus aureus*. Of these, the *K. pneumoniae* isolate came from a patient on antimicrobial therapy.

The color intensity of the filter cards was 3+ to 4+ for 74.0% of the pure pathogens (60 of 81), with the remaining 26.0% (21 to 81) in the 1+ to 2+ range. The remaining 140 cultures in group 1 represented pure cultures of contaminants or mixed cultures containing two or more organisms. Of these, the Bac-T-Screen detected 57.1% (80 of 140).

(ii) Autobac. Of the 246 specimens in group 1, 63% (155 of 246) were detected by the Autobac system (Table 1). Considering the 106 pure cultures of probable pathogens, the Autobac system detected 90.6% (96 of 106). Those organisms not detected by the Autobac included four isolates of yeast, five gram-negative rods from patients on antimicrobial therapy, and one isolate of *S. aureus*.

Group 2. The Bac-T-Screen detected 19.5% of the specimens in group 2 (23 of 118); the Autobac detected 22.9% (27 of 118) (Table 1). In addition, group 2 included two specimens which clogged the filter and 11 which left a residual pigment. There were 24 pure cultures of probable pathogens. The Bac-T-Screen detected 37.5% of the gram-negative rods (9 of 24); the Autobac system detected 50%. A majority gave a +/− reaction on the Bac-T-Screen filter card, which was interpreted as negative. Both of the urine specimens clogging the filter in this group contained WBCs and a pure pathogen.

Group 3. Of the 636 specimens in group 3, 72.6% (462 of 636) were negative by Bac-T-Screen. Of the remaining cultures, 13.8% (88 of 636) were detected as positive by Bac-T-Screen, and 13.6% (86 of 636) were uninterpretable. The majority of the false-positive Bac-T-Screen filter cards had a color intensity of 1+. Within this group, 97.3% (619 of 636) were negative by the Autobac system after 4 h of incubation.

Uninterpretable specimens. A total of 13.3% of the specimens in this study (133 of 1,000) were uninterpretable by the Bac-T-Screen owing to clogging of the filter (36 specimens) or a residual pigment on the card (97 specimens). Of the urine specimens that left a residual pigment on the filter card, a majority (90.7%) were negative cultures with colony counts of $<10^5$ CFU/ml. Only 2.1% of the specimens (2 of 97) contained pure cultures of probable pathogens with colony counts of $\geq 10^5$ CFU/ml. Of the 97 pigmented urine specimens, 51.5% (50 of 97) had a dark-yellow-to-brown color when examined macroscopically. Overall, 89.6% (87 of 97) had a positive urinalysis dip stick for blood, bilirubin, urobilinogen, or erythrocytes seen on microscopic examination of the urine sediment. The pigmented urine specimens represented 3.6% of group 1 cultures, 9.3% of group 2, and 12.1% of group 3 (Table 1).

Of the specimens which clogged the Bac-T-Screen filter card, 69.4% (25 of 36) were in group 1, 5.6% (2 of 36) were in group 2, and 25.0% (9 of 36) were in group 3. Overall, the urine specimens clogging the filter represented 10.2% of the group 1 specimens, 1.7% of group 2, and 1.4% of group 3 (Table 1). The majority of those clogging the filter contained large amounts of protein and WBCs.

Predictive value. In the data analyses, those specimens clogging the Bac-T-Screen filter were considered positive, and those leaving a residual pigment were considered negative. Table 2 shows the positive and negative predictive values for Bac-T-Screen and the Autobac urine screen. The data shown were calculated in two ways, depending upon the definition of significant bacteriuria. One definition considered all urine cultures positive with colony counts of $\geq 10^5$ CFU/ml regardless of the organisms present. Since the screening methods cannot differentiate the types and numbers of organisms present, this analysis was necessary. However, since a true definition of significant bacteriuria is a pure culture of a probable pathogen with a colony count of $\geq 10^5$ CFU/ml, analyses of these specimens are also presented. When the 246 urine specimens containing $\geq 10^5$ CFU/ml are considered, the Bac-T-Screen and Autobac are comparably sensitive (75.6 versus 75.2%); however, the Autobac system is more specific (94.2%) than the Bac-T-Screen (83.8%). When only urine specimens containing a pure culture of a probable pathogen with $\geq 10^5$ CFU/ml are considered, the sensitivity of Bac-T-Screen is

TABLE 2. Predictive values for Bac-T-Screen and Autobac

Parameter	Predictive value (%)			
	Bac-T-Screen		Autobac	
	All organisms	Pure pathogens	All organisms	Pure pathogens
Sensitivity	75.6	93.4	75.2	90.6
Specificity	83.8	76.6	94.2	88.5
Predictive value positive	60.4	32.1	77.9	48.2
Predictive value negative	91.3	99.0	88.6	98.7

93.4% compared with 90.6% for Autobac. Since the false-positive rate for Bac-T-Screen is higher than that for the Autobac (16.2 versus 5.8%), the Autobac is more specific in predicting a positive. The ability of Bac-T-Screen to detect a negative urine specimen (99.0%) is comparable to that of Autobac (98.7%).

DISCUSSION

The main purpose of urine screening is to rapidly detect urine specimens which do not contain significant bacteriuria. The ability of a method to identify these specimens is of major importance since approximately 70 to 80% of urine specimens received for culture in a general hospital are negative (5, 9, 13). Both urine screening methods evaluated in this study provide rapid results. However, the major difference between the two systems is that results were obtained within 2 min by Bac-T-Screen, as compared with 1 to 4 h by the Autobac system. If we consider only $\geq 10^5$ CFU/ml of a single organism in pure culture, each method has a 99% probability of predicting a negative urine specimen. The sensitivities of the methods are 93.4% (including cloggers) for Bac-T-Screen and 90.6% for Autobac.

The problem of specimens uninterpretable by Bac-T-Screen may be of concern since these specimens require plating for interpretation. In this study, 13.3% of the study population could not be interpreted. However, since a majority of those clogging the filter contained WBCs and had colony counts of $\geq 10^5$ CFU of a single organism per ml in pure culture, these were considered positive specimens in the data analyses.

A majority of those specimens leaving residual pigment on the filter card were negative by the culture method and therefore were interpreted as negative in this study. However, 9.3% (9 of 97) had colony counts of $\geq 10^5$ CFU/ml, and 2.1% were probable pathogens. Since these were uninterpretable by Bac-T-Screen, they should probably have been plated and therefore been considered as positive specimens. If they had been considered positive, the false-positive rate of Bac-T-Screen would have increased, and the specificity and predictive value positive would have decreased.

The Bac-T-Screen detected all of the yeast isolates in this study and all but one of the isolates from patients on antimicrobial therapy. Previous studies have reported a high false-negative rate for these organisms when instrumentation is used for urine screening (4, 6). Most of the other rapid methods require growth to occur before a positive result is detected, and therefore, slow-growing organisms or organisms allowed to incubate in the presence of antimicrobial agents may not be detected within the 4- to 13-h incubation time. The Bac-T-Screen differs from most other screening methods in that a detection of a positive is dependent on the presence of organisms in the urine when tested, and therefore, incubation of the specimen is unnecessary.

In this study, the Bac-T-Screen detected the four yeast isolates within 2 min, whereas none were detected by Autobac. Similar findings have been reported by others for the Autobac system (3, 4, 6). In one study, no yeast was detected after 3 h by Autobac; however, the detection rate increased to 87.5% (7 of 8) after 6 h of incubation (6). If incubation had been continued for 6 h, the false-negative rate for yeast may have decreased. However, Hale et al. reported that as incubation time for Autobac increased from 4 to 6 h, the false-positive rate also increased (4.1 to 13.9%) (4). If maximum incubation (6 h) is used, the false-positive rate of Bac-T-Screen and Autobac would probably be comparable.

In conclusion, the most important contribution of the Bac-T-Screen is its ability to obtain results within 2 min. It can also detect yeast as well as organisms from patients on antimicrobial agents, situations which have presented problems with other screening methods. Its sensitivity and its ability to predict a negative urine specimen were comparable to those of the Autobac in this study and to other automated screening methods previously described (13). It also has the advantages of being simple to operate and of requiring a small amount of laboratory space. Use of the Bac-T-Screen to screen clean-voided urine specimens will provide an overall time saving to clinical microbiology laboratories as well as an advantage to physicians and to the patients who will receive more prompt care.

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