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Evaluation and Optimization of Urine Screening by Autobac

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The purpose of this investigation was to evaluate the effectiveness of the Autobac (Pfizer Inc., New York, N.Y.) urine screen for detection of bacteriuria in 3,026 urine specimens and to establish the optimum procedure for the Autobac system. Overall, 97% of urine specimens having $>10^5$ colony-forming units (CFU) per ml were detected within 5 h by the Autobac system. The system detected 66, 90, and 94% of such specimens after 2, 3, and 4 h of incubation, respectively. Of specimens having 10^4 to 10^5 CFU/ml, the Autobac system detected 10, 45, 53, and 95% after 2, 3, 4, and 5 h of incubation, respectively. The rate of false-positive results increased from 0% after 2 h to 2% after 3 h to 6% after 4 h and 25% after 5 h of incubation. The specificity of the urine screening results also varied with the incubation time. Percentages of specimens having $>10^5$ CFU/ml that gave positive urine screening results at various times were as follows: 96% at 2 h, 74% at 3 h, 29% at 4 h, and 9% at 5 h. These findings suggest that a 3- or 4-h urine screening procedure will effectively detect bacteriuria of $>10^5$ CFU/ml, with few false-positive results. However, a 5-h procedure, which gives more false-positive results, may be needed for detection of lower levels of bacteriuria.

Urine cultures represent a major portion of the work load of most clinical microbiology laboratories. By standard procedures, each specimen must be cultured, and any organisms isolated must be further processed before results can be reported. This procedure requires 24 to 48 h for completion, and urine culture results are often issued too late to be of prospective clinical value. In addition, the same culture procedure is applied to approximately 80% of the urine specimens that are negative for significant numbers of potentially pathogenic organisms, which decreases the efficiency of operation of laboratories. Urine screening methods provide for rapid detection of significant bacteriuria, and they eliminate the need for processing negative specimens. Thus, urine screening may allow more rapid reporting of urine culture results and improve the efficiency of operation of laboratories.

Several methods for urine screening have been proposed, including manual procedures (2) and automated systems (3, 5). The Autobac system (Pfizer Inc. New York, N.Y.) provides a semiautomated approach to urine screening, based on the detection of changes in light scattering due to growth of organisms in broth inoculated with urine. The Autobac instrument has been reported to provide an effective urine screening system, but the optimum procedure for Autobac urine screening has not been established (4, 7). The purpose of this investigation was to evaluate the effectiveness of the Autobac urine screening procedure for detection of bacteriuria in 3,026 urine specimens and to establish the optimum conditions for urine screening with the Autobac system.

MATERIALS AND METHODS

Specimens. The University of Texas Medical Branch provided 3,026 urine specimens collected over a 3-month period. Specimens obtained from infants or from patients with indwelling catheters were excluded from the study, but all other types of specimens were analyzed. The specimens were processed immediately upon receipt in the laboratory. Each specimen was first cultured by standard methods and then processed for urine screening.

Quantitative urine culture. All urine specimens were streaked with a 0.01-ml calibrated loop onto sheep blood agar and MacConkey agar plates, and colony counts were determined after 18 to 24 h of incubation (1). Viridans group streptococci, diphtheroids, and lactobacilli were considered to be normal flora contaminants, but all other organisms were regarded as potential urinary pathogens.

Urine screening. Urine screening was performed with Autobac 1, an instrument composed of an incubator-shaker and a photometer module for measurements of light scattering. For urine screening, the instrument was operated in the calibrate mode which generates voltage readings. Growth of an organism is reflected by a decrease in the voltage reading. Autobac

cuvettes were filled with 18 ml of low-thymidine Eugonic broth (Pfizer Inc.), and each cuvette chamber received approximately 1.4 ml of broth. Individual cuvette chambers were inoculated with 0.1 ml of urine specimen by using a micropipetting device with disposable tips (Pipetman, Gilson Scientific, Middleton, Wis.). The cuvettes were incubated at 37°C, with rotation at 220 rpm for 15 min to allow for equilibration and mixing of the inoculum into the broth. A base-line voltage reading was then made for each cuvette chamber, and the cuvettes were returned to the incubator-shaker. Additional readings were taken at intervals, and the voltage drop was calculated by subtracting the base-line reading from the final reading. A voltage decrease of >0.2 V was considered to be a positive result (7). The study was carried out in three separate phases: an initial evaluation of 1,037 specimens by using 5-h of urine screening, an evaluation of 486 specimens by using 2, 3, and 5 h of urine screening, and an evaluation of 1,503 specimens by using 3 and 4 h of urine screening.

RESULTS

Preliminary evaluation of urine screening by Autobac. A total of 1,037 urine specimens were analyzed by standard culture methods compared with the Autobac urine screening procedure. In this initial study, voltage changes were recorded after 5 h of incubation. Potential urinary pathogens in quantities $>10^5$/ml were recovered by standard culture methods from 127 specimens. Of these specimens, 123 (97%) were also positive by the Autobac urine screening procedure. Sixty specimens were found by standard culture methods to have 10^4 to 10^5 organisms per ml, and 54 (90%) of these specimens gave positive urine screening results. Organisms present in quantities $>10^4$/ml that were not detected by the urine screening procedure included four *Streptococcus agalactiae*, three coagulase-negative staphylococci, and three gram-negative bacilli (*Escherichia coli*, *Citrobacter* species, and *Providencia alcalifaciens*).

In this initial evaluation, 365 specimens gave positive Autobac screening results. Of these Autobac-positive specimens, 177 had $>10^4$ organisms per ml, as described above, but an additional 125 specimens had $<10^4$ organisms per ml, and 63 had no growth in standard urine cultures. Therefore, of the 365 specimens reported to be positive by a 5-h Autobac procedure, 188 (51%) were false-positive. This high rate of false-positive results led to the following investigations into the effectiveness of shorter incubation times in the Autobac urine screening procedure.

Effect of incubation time on Autobac urine screening. The effectiveness of varied periods of incubation in the detection of significantly positive urine specimens and the reduction of false-positive results was investigated by using 486 urine specimens. These specimens were analyzed by standard culture methods and compared with the Autobac urine screening procedure, with voltage changes recorded after 2, 3, and 5 h of incubation. Potential urinary pathogens in quantities of $>10^5$/ml were detected in 73 specimens by standard culture methods (Table 1). Of these specimens, 66% were positive by the Autobac procedure after 2 h of incubation, 90% were positive after 3 h, and 94% were positive after 5 h of incubation. Of 19 specimens that had between 10^4 and 10^5 organisms per ml, 10% were positive by the Autobac screening procedure after 2 h, 37% were positive after 3 h, and 95% were positive after 5 h. By standard culture methods 83 specimens produced $<10^4$ colony-forming units (CFU)/ml. Of these specimens, none were positive by the screening procedure after 2 h of incubation, 2% were positive after 3 h, and 25% were positive after 5 h.

Effectiveness of a 3- or 4-h incubation in the Autobac urine screening procedure. A total of 1,503 urine specimens was analyzed by urine screening with readings taken after 3 or 4 h of incubation, and the results were compared with colony counts obtained from standard urine culture methods (Table 2). By standard culture methods, 104 specimens had colony counts of $>10^5$ organisms per ml, 43 had colony counts of 10^4 to 10^5 per ml, and 368 had counts of $<10^4$ organisms per ml.

The urine screening results after 3 h of incubation were similar to those presented above; 89% of the specimens with $>10^5$ CFU/ml were positive, 49% of the specimens with 10^4 to 10^5 CFU/ml were positive, and only 3% of the specimens with $<10^4$ CFU/ml were positive. After 4 h of incubation, 94% of the specimens with colony counts of $>10^5$/ml were detected by the screening procedure, and 53% of the specimens

TABLE 1. *Detection of bacteriuria by urine screening after 2, 3, or 5 h of incubation in Eugonic broth*

CFU/ml[a]	No.[b]	% of specimens positive after the following incubation time[c]		
		2 h	3 h	5 h
$>10^5$	73	66	90	94
10^4-10^5	19	10	37	95
$<10^4$	83	0	2	25

[a] CFU/ml of urine as determined by standard quantitative cultures.

[b] Number of urine specimens with the indicated colony count. Total specimens analyzed, 486.

[c] Time of incubation of Autobac urine screening procedure. Results are expressed as cumulative percent of specimens positive by urine screening.

TABLE 2. *Efficiency of a 4-h incubation for detection of bacteriuria by urine screening*

CFU/ml[a]	No.[b]	% of specimens positive after the following incubation time[c]	
		3 h	4 h
$>10^5$	104	89	94
10^4-10^5	43	49	53
$<10^4$	368	3	6

[a] CFU/ml of urine as determined by standard quantitative cultures.

[b] Number of urine specimens with the indicated colony count. Total specimens analyzed, 1,503.

[c] Time of incubation of Autobac urine screening procedure. Results are expressed as cumulative percent of the specimens positive by urine screening.

TABLE 3. *Correlation of colony count and incubation time required for positive urine screening*

Incubation time (h)[a]	No. of positive specimens[b]	% of specimens positive with the following CFU/ml[c]		
		$>10^5$	10^4-10^5	$<10^4$
2	50	96	4	0
3	150	74	18	8
4	17	29	12	59
5	33	9	33	58

[a] Length of incubation in the Autobac urine screening procedure.

[b] Number of positive specimens at the indicated incubation time. Total specimens analyzed, 1,989.

[c] CFU/ml of urine as determined by standard quantitative cultures. Results are expressed as the percent of specimens positive after the indicated incubation times that had the indicated colony counts.

with 10^4 to 10^5 CFU/ml were detected. The false-positive rate remained low; only 6% of the specimens with a colony count of $<10^4$/ml were positive by the screening procedure after 4 h of incubation.

Influence of urine culture colony count on the incubation time required for a positive urine screening result. Analysis of the composite data obtained from the two urine screening experiments presented above was done to determine the predictive value of urine screening results (Table 3). A total of 50 specimens had positive urine screening results after 2 h of incubation. Of these specimens, 96% had colony counts of $>10^5$/ml, and 4% had colony counts of 10^4 to 10^5/ml. No specimens with colony counts of $<10^4$/ml were positive after 2 h of incubation. Of 150 specimens that had positive urine screening results after 3 h of incubation, 74% had colony counts of $>10^5$/ml, 18% had colony counts of 10^4 to 10^5/ml, and 8% had colony counts of $<10^4$/ml. After 4 h of incubation, 17 specimens were positive by urine screening. Of these specimens, only 41% had colony counts of $>10^4$/ml, and 59% had colony counts of $<10^4$/ml. Of 33 specimens that had positive urine screening results after 5 h of incubation, 9% had colony counts of $>10^5$/ml, 33% had colony counts of 10^4 to 10^5/ml, and 58% had colony counts of $<10^4$/ml.

DISCUSSION

Our preliminary analysis of the Autobac urine screening procedure with a 5-h incubation time confirmed the findings of others (4, 7) that this system is effective for the detection of significant bacteriuria. The Autobac procedure detected 95% of urine specimens with colony counts of $>10^4$/ml. However, significant numbers of false-positive results were also obtained, and these findings caused us to investigate the utility of shorter incubation times in the screening procedure.

The results of studies to investigate the effect of incubation time suggest that the specificity of urine screening for the detection of significant bacteriuria is inversely proportional to the incubation time, whereas the sensitivity of detection of bacteriuria is directly proportional to the incubation time. Urine screening for 2 h produced highly specific results; 100% of positive specimens had $>10^4$ CFU/ml. However, the sensitivity of detection of bacteriuria was low; only 66% of specimens with $>10^5$ CFU/ml and 10% of specimens having 10^4 to 10^5 CFU/ml were detected. A 3-h urine screening procedure detected 90% of specimens with $>10^5$ CFU/ml but only 45% of specimens with 10^4 to 10^5 CFU/ml. The 3-h screening results demonstrated a high degree of specificity; 92% of specimens positive at 3 h had $>10^4$ CFU/ml. The specificity of urine screening results decreased with a 4- or 5-h incubation. Only 41% of specimens positive at 4 h had $>10^4$ CFU/ml, and only 42% of specimens positive at 5 h had $>10^4$ CFU/ml. However, the sensitivity of the urine screening results increased with these longer incubations. The 4-h screening procedure detected 94% of specimens with $>10^5$ CFU/ml and 53% of specimens with 10^4 to 10^5 CFU/ml. The 5-h procedure detected 95% of specimens with $>10^4$ CFU/ml.

Although the sensitivity of the urine screening procedure increases with the incubation time, the number of false-positive urine screening results also increases with longer incubation times. There were no false-positive results after 2 h of urine screening, and only 8% false-positive results were encountered after 3 h of urine screening. However, nearly 60% of the specimens that

became positive after 4 or 5 h of incubation were false-positive, and use of a 5-h urine screening procedure without any earlier readings resulted in about 50% false-positive results. Such results may be misleading to physicians, and they produce unnecessary work in laboratories.

In previous studies, Thrupp et al. (7) revealed that 44% of urine specimens with $>10^5$ CFU/ml of gram-negative bacilli gave positive urine screening results after only 1 h of incubation, and 81% of such specimens were positive after 2 h of urine screening. Gram-positive organisms were detected only after 3 or 4 h of incubation. These studies suggested that a 3-hour screening procedure would detect more than 90% of specimens having significant bacterial counts. However, Jenkins et al. (4) found that only 75% of specimens with $>10^5$ CFU/ml were detected after 3 h, and 95% of such specimens were detected only after 6 h of urine screening. This discrepancy may have been due to the limited number of specimens analyzed (7) or to refrigeration of the specimens before analysis (4). Our findings extend those reported previously and suggest that a 3- or 4-h urine screening procedure will detect at least 90% of significantly positive urine specimens.

Our observations suggest that several variations of the Autobac procedure may be used, depending on the goals to be accomplished by urine screening. If urine screening is used to rapidly detect urine specimens with $>10^5$ CFU/ml, a 3- or 4-h Autobac procedure may be used. A 3-h procedure will detect about 90% of such specimens with a low level of false-positive results, and the results can be reported within a short time after receipt of the specimen. In addition, specimens positive after 3 h of screening can be processed for rapid identification and susceptibility testing (4). A 4-h urine screening procedure will slightly improve the rate of detection to 94%. However, the rate of false-positive results is also slightly increased after 4 h of incubation, and it would be difficult to perform rapid identification and susceptibility testing within an 8-h working day for specimens positive after 4 h of urine screening.

If the goal of urine screening is to detect urine specimens having $>10^4$ CFU/ml, a 5-h Autobac procedure should be used. Screening procedures of 3 or 4 h detected only about 50% of specimens having 10^4 to 10^5 CFU/ml, whereas the 5-h procedure detected 95% of such specimens. Our results suggest that longer screening procedures not are indicated, because a 5-h incubation provided the same rate of detection as did the 6-h procedure previously reported (4). An alternative approach is to use a 3-h screening procedure for rapid detection of significantly positive specimens coupled with a standard culture on a blood agar plate or a biplate for detection of specimens that are not positive by urine screening. Such an approach takes advantage of the rapid reporting capability of the urine screening procedure while maintaining optimum accuracy in the detection of positive urine specimens. Use of a standard culture also allows for detection of low level bacteriuria which may be significant in some cases (6).

In summary, our studies have shown that the Autobac instrument provides an effective and versatile system for urine screening. The length of the screening procedure has a significant effect on the utility of the system, and the occurrence of false-positive and false-negative results varies with incubation time. Short incubation times in urine screening procedures result in decreased false-positive results but increased false-negative results. Longer incubation times produce few false-negative results but more false-positive findings. The results indicate that a 3- or 4-h screening procedure is optimal for the efficient detection of urine specimens with $>10^5$ CFU/ml, but a 5-h procedure is needed for reliable detection of specimens with 10^4 to 10^5 CFU/ml. These findings suggest that the Autobac urine screening procedure may be adjusted to meet the needs of individual laboratories. Overall, our findings indicate that urine screening is a valuable procedure that can provide more rapid reporting of urine culture results and increased efficiency of operation of microbiology laboratories.

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