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Rapid Screening for Bacteriuria by Light Scatter Photometry (Autobac): a Collaborative Study

DEVON C. HALE,[1]* DONALD N. WRIGHT,[2]† JAMES E. MCKIE,[3] HENRY D. ISENBERG,[4] RONALD D. JENKINS,[2] AND JOHN M. MATSEN[2]

Riverview Hospital, Idaho Falls, Idaho 83401,[1] University of Utah Medical Center, Salt Lake City, Utah 84132,[2] Pfizer Diagnostics, Groton, Connecticut 06340,[3] and Long Island Jewish-Hillside Medical Center, New Hyde Park, New York 11040[4]

A total of 2,720 urine specimens from three laboratories were evaluated by Autobac (Pfizer Diagnostics) and were compared with simultaneous colony counts for evidence of bacteriuria. Of 599 specimens with a colony count of $\geq 10^5$ colony-forming units per ml, 93.8% were detected within 6 h. This detection rate increased to 97% of 447 positive urine specimens when only specimens from patients not on antimicrobials were evaluated. The majority (77.9%) of positive specimens were detected as early as 3 h. Those specimens with $\geq 10^5$ colony-forming units per ml, which were negative by Autobac at 6 h, included organisms which are frequently considered to be contaminants (diphtheroids, lactobacilli, alpha and gamma streptococci, yeasts, and *Staphylococcus epidermidis*), or were from patients who were being treated with antimicrobial agents. Of 2,121 urine specimens with colony counts of $<10^5$, 98.1% were correctly determined to be negative by Autobac at 3 h. This percentage decreased to 86.0 at 6 h. The majority of these false-positive specimens were those with colony counts of 10^4 to 10^5 colony-forming units per ml. There appeared to be no major difference in results from the three laboratories or among the four lots of broth used in this study.

Recent papers (2, 3) have reported the effectiveness of using light scatter photometry to detect bacteriuria. A multicenter collaborative study was established to substantiate these reports by using clinical specimens in a comparison between a light-scattering system (Autobac; Pfizer Diagnostics) and a conventional, quantitative colony-count technique. This study evaluated the reliability of forward light scattering in detecting bacteriuria, interlaboratory reproducibility, and variation among four different broth lots.

MATERIALS AND METHODS

Specimens. At each of the three participating centers (Long Island Jewish-Hillside Medical Center, Riverview Hospital, and University of Utah Medical Center), clinical urine specimens were obtained from the clinical microbiology laboratories. For each specimen entering the study, the following information was requested: the method of collection (clean catch, catheter, or pediatric bag), the time of collection, and any antimicrobial therapy the patient was receiving. The urine specimens were stored at 4°C for not more than 24 h (1, 4) before processing. Specimens were simultaneously examined by light-scattering (Autobac) and quantitative loop methods (1), and the results were compared.

† Present address: Department of Microbiology, Brigham Young University, Provo, UT 84602.

Instrumentation. The Autobac system (5-7) consisted of a photometer and electronics, an incubator-shaker, and a multichambered optical cuvette. The photometric system converted the light scattered by the organisms into a voltage reading indicative of the turbidity of the suspension. If bacterial growth occurred, light scattering increased, which resulted in a voltage reading change. The incubator-shaker provided incubation of the cuvettes at 36°C with continuous agitation at 220 rpm. The cuvette used was the standard unit supplied with the Autobac system containing 12 sample chambers accessible via stoppered ports above each individual chamber.

Media. For this Autobac procedure, each urine sample was simultaneously inoculated into corresponding chambers of four cuvettes, each containing a different lot of Eugonic broth (Pfizer Diagnostics). The four broth lots were used to examine lot-to-lot reproducibility and consisted of three lots of regular Eugonic broth (7Y558, 83560, and 84574) and one lot of low-thymidine Eugonic broth (85551). MacConkey agar (BBL Microbiology Systems; HOJ-4382; expiration date, April 1982) and Trypticase soy blood agar (BBL; D1D-HNP; expiration date, April 1982) were used for the conventional quantitative loop method. Media from the same lots were used by all three participating centers throughout the study.

Control organisms. *Streptococcus faecalis* (ATCC 065-02A), *Escherichia coli* (ATCC 29194), and *Pseudomonas aeruginosa* (ATCC 27853) were used as control organisms. By using the inoculum standardization meter of the Autobac photometer (5-7), sus-

pension (1.5×10^7 to 3.0×10^7 colony-forming units (CFU) per ml) of each control organism was prepared in 0.9% NaCl. These suspensions were then diluted 1:10, 1:100, and 1:1,000 with 0.9% NaCl.

Autobac procedure. Autobac urine screening was performed by distributing 18 ml of Eugonic broth among the 12 cuvette chambers and a control chamber as previously described (5-7). Each urine specimen was mixed well, and 0.1 ml was inoculated into the broth contained in 1 of the 12 sample chambers. After the sample chambers of the cuvette were inoculated, they were sealed with a gasket supplied with the cuvette. The inoculated cuvettes were placed into the incubator-shaker and incubated for 15 min. A base-line light scatter voltage (LVS) at time zero ($T = 0$) for each of the 12 sample chambers ($n_1, n_2 \ldots n_{12}$) was then obtained by placing the cuvette in the photometer with the photometer in the calibration mode. A reporting ticket was used in the standard manner to initiate the automatic reading of the base-line voltage for each chamber. The cuvette was again placed in the incubator-shaker, with subsequent voltage readings taken at 3, 4, 5, and 6 h (e.g., $LSV^{n}_{T=3} \ldots LSV^{n}_{T=6}$). After each incubation period, the voltage obtained with each chamber was compared with the base-line voltage of that chamber, and a voltage change of ≥0.20 V was regarded as positive. A sample in chamber one, giving a base-line reading ($LSV^{n=1}_{T=0}$) of 3.20 V and a reading ($LSV^{n=1}_{T=3}$) of 3.00 V at 3 h was considered positive because the voltage change was ≥0.20 V.

Reference procedure. A .001-ml loop calibrated by the procedure of Barry et al. (1) was used to streak each urine sample onto a Trypticase soy blood agar plate and a MacConkey agar plate. The colony count was determined at 18 to 24 h as previously described (1). For the purpose of statistical analysis, $\geq 10^5$ CFU/ml was considered significant.

RESULTS

Detection of positive specimens. A total of 2,720 clinical urine specimens were evaluated. Of these, 599 (22.0%) had a colony count of $\geq 10^5$ CFU/ml. At 3 h, 77.9% (Table 1) of the specimens with a colony count of $\geq 10^5$ CFU/ml were determined to be positive by the Autobac method. This detection rate increased to 93.8% after 6 h of incubation. Table 2 shows a breakdown by organism of positive urine specimens detected by Autobac. Of 316 urine specimens containing $\geq 10^5$ CFU of *E. coli* per ml, 25 were missed at 3 h, 10 were missed at 4 h, and 4 were missed at 5 and 6 h. Of urine specimens containing significant numbers of gram-negative rods, 88.7% were detected at 3 h, 93.5% were detected at 4 h, 97.6% were detected at 5 h, and 97.9% were detected at 6 h. Enterococci were the predominant gram-positive organisms isolated. At 3 h, 17 of 46 specimens containing $\geq 10^5$ CFU of enterococci (63%) per ml had not been detected, but at 6 h, all had been detected. Of 30 specimens

TABLE 1. *Ability of Autobac to detect positive and negative urine specimens*

Time (h)	% of 599 positive[a] urine specimens positive by Autobac	% of 2,121 negative[b] urine specimens negative by Autobac
3	77.9	98.1
4	86.7	95.8
5	91.5	91.5
6	93.8	86.0

[a] $\geq 10^5$ CFU/ml by reference method at 18 to 24 h.
[b] $<10^5$ CFU/ml by reference method at 18 to 24 h.

TABLE 2. *Number of positive urine specimens containing organisms not detected at each time period by Autobac*

Principal organism	Total no. of specimens	No. of specimens[a] at:			
		3 h	4 h	5 h	6 h
Gram-negative rods					
Acinetobacter species	2 (2)	0 (0)	0 (0)	0 (0)	0 (0)
Citrobacter species	5 (5)	0 (0)	0 (0)	0 (0)	0 (0)
Enterobacter species	12 (10)	3 (0)	2 (0)	1 (0)	1 (0)
Escherichia coli	316 (246)	25 (9)	10 (0)	4 (0)	4 (0)
Klebsiella species	48 (31)	4 (2)	1 (0)	1 (0)	1 (0)
Proteus mirabilis	38 (29)	10 (0)	10 (0)	1 (0)	1 (0)
Other *Proteus* species	8 (7)	3 (1)	2 (0)	1 (0)	1 (0)
Providencia species	3 (1)	0 (0)	0 (0)	0 (0)	0 (0)
Pseudomonas aeruginosa	26 (14)	7 (4)	5 (2)	3 (0)	3 (0)
Other *Pseudomonas* species	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)
Serratia species	3 (1)	0 (0)	0 (0)	0 (0)	0 (0)
Gram-positive cocci					
Enterococci	46 (37)	17 (6)	5 (0)	3 (0)	0 (0)
Staphylococcus aureus	13 (12)	3 (1)	1 (0)	0 (0)	0 (0)
Staphylococcus epidermidis	30 (21)	25 (11)	20 (9)	10 (4)	5 (3)
Streptococcus agalactiae	3 (3)	0 (0)	0 (0)	0 (0)	0 (0)
Streptococcus pyogenes	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)
Streptococcus viridans	11 (9)	10 (5)	7 (4)	4 (3)	3 (2)
Other *Streptococcus* species	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)
Other gram-positive cocci	1 (1)	1 (1)	1 (1)	0 (0)	0 (0)
Gram-positive rods					
Bacillus species	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)
Diphtheroids	5 (5)	4 (4)	3 (3)	2 (2)	1 (1)
Lactobacillus species	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)
Unidentified organisms	6 (4)	2 (0)	1 (0)	1 (0)	1 (0)
Yeasts					
Candida albicans	9 (0)	9 (0)	9 (0)	9 (0)	8 (0)
Other species of yeasts	9 (4)	9 (4)	9 (4)	8 (4)	7 (4)

[a] Numbers within parentheses indicate a subpopulation of positive urine specimens from patients taking no antimicrobial therapy.

containing $\geq 10^5$ CFU of *Staphylococcus epidermidis*, 25 (84%) were not detected at 3 h, 20 (67%) were not detected at 4 h, 10 (33%) were not detected at 5 h, and 5 (17%) were not detected at 6 h. Other organisms that were occasionally missed at 6 h included diphtheroids, alpha streptococci, and yeasts. Some species of these organisms do not grow well in Eugonic broth, which may account for the failure of the Autobac to detect them. Analysis of results of 752 urine specimens from one of the three laboratories indicated that the method of specimen collection had no effect on the ability of the Autobac to detect positives.

Data from patients not on antimicrobials. A total of 1,940 specimens were obtained from the patients whose records indicated no antimicrobial therapy. Of this group, a total of 447 (23.0%) were positive by the reference method after 18 to 24 h of incubation. Of these positive specimens (Table 3), 88.8% were detected within 3 h by the Autobac procedure; after 6 h, 97.3% of the positive specimens were recognized. The numbers in parentheses in Table 2 indicate the number of specimens containing $\geq 10^5$ CFU/ml (from the group of patients not on antimicrobials) that were not detected at each reading time. All specimens containing gram-negative rods or enterococci, which were positive by the reference method, were detected by the Autobac procedure.

TABLE 3. *Ability of Autobac to detect positive and negative urine specimens from patients not receiving antimicrobial agents*

Time (h)	% of 447 positive[a] urine specimens positive by Autobac	% of 1,493 negative[b] urine specimens negative by Autobac
3	88.8	98.1
4	94.0	95.4
5	96.6	90.9
6	97.3	85.6

[a] $\geq 10^5$ CFU/ml by reference method in 18 to 24 h.
[b] $<10^5$ CFU/ml by reference method in 18 to 24 h.

TABLE 4. *Profile of false-positive specimens*

Total colony count by reference method[a] (CFU/ml)	No. (incidence [%]) of false-positive specimens[b] after following time of incubation by the Autobac method			
	3 h	4 h	5 h	6 h
10^4-10^5	17 (0.8)	61 (2.9)	117 (5.5)	167 (7.9)
10^3-10^4	2 (0.1)	3 (0.1)	29 (1.4)	63 (3.0)
$<10^3$	19 (0.9)	23 (1.1)	33 (1.5)	64 (3.0)
Total false-positive specimens[c]	38 (1.8)	87 (4.1)	179 (8.4)	294 (13.9)

[a] Surface-streak/calibrated-loop method (1).
[b] Positive by Autobac method and negative by reference method.
[c] Total negative specimens (i.e., $<10^5$ CFU/ml) = 2,121.

TABLE 5. *Untreated[a] urine specimens with colony counts of 10^4 to 10^5 detected by Autobac at 6 h*

Organism	No. (%) of specimens	
	Reference method	Autobac
Gram-negative rods	81	76 (93.8)
Enterococci	22	18 (81.8)
Other organisms[b]	71	32 (45.1)

[a] Urine specimens from patients known to be not receiving antimicrobial therapy at the time of specimen collection.
[b] Primarily *S. epidermidis*, nonenterococcal Streptococci, and *Bacillus* spp.

Detection of true-negative specimens. At 3 h, 1.9% of urine samples with $<10^5$ CFU/ml were detected as positive by the Autobac (Table 1). The number of false-positive specimens continued to increase during the 6 h of incubation, ultimately reaching a total of 14%. The majority (45% at 3 h, 70% at 4 h, 65% at 5 h, and 57% at 6 h) (Table 4) of these false-positive specimens contained 10^4 to 10^5 CFU/ml. Table 5 shows data from untreated patients whose urine specimens contained 10^4 to 10^5 CFU/ml and illustrates that 93.8% of specimens containing gram-negative rods and 81.8% containing enterococci in this range were detected as positive by the Autobac after 6 h of incubation. Other organisms, primarily *S. epidermidis*, nonenterococcal streptococci, and *Bacillus* species were detected only 45.1% of the time in this range of bacterial density.

Controls. Controls demonstrated that the detection of bacterial growth was consistent on a repetitive basis.

Broth-to-broth variation. All broth lots supported bacterial growth equally well, and small variations in detection times and numbers appeared to occur in a random manner.

DISCUSSION

The Autobac procedure has been particularly impressive in its ability to detect bacteriuria caused by gram-negative rods and enterococci. Since the majority of urinary tract infections are caused by these organisms, it is particularly important that the Autobac detected these bacteria efficiently. Of the 508 specimens containing $\geq 10^5$ CFU of these bacteria per ml, 497 (97.8%) were detected by the Autobac (Table 2). All 382 (100%) specimens from untreated patients, which were positive by the manual method, were also positive by the Autobac. This is consistent

with the study by Jenkins et. al. (3) in which five specimens with significant numbers of gram-negative rods, missed by the Autobac, were obtained from patients receiving antimicrobial therapy effective against the organism. The dilution of urine antimicrobial content in the automated assay is only 1:15 and is kept constant, whereas the smaller volume applied by the loop diffuses rapidly into a much larger volume of agar, decreasing drug concentration in contact with the bacteria rapidly, and may account for the difference in detection of bacteria in these two procedures.

At 6 h, a false-positive sample rate of 13.9% was demonstrated by the stringent criteria applied; 57% of these false-positive specimens contained 10^4 to 10^5 CFU/ml, a bacterial density of potential significance to some clinicians.

The Autobac urine screening procedure can be used in the clinical laboratory in at least two different ways. It may serve as a screening procedure, consisting of an initial base-line reading followed by one additional reading after 5 or 6 h of incubation. All negative results could be reported. Specimens that are positive could be processed routinely. A variation of this procedure consists of an initial base-line and a 3-h Autobac reading, leading to prompt recognition of important bacteria with minimal delay. Such positive results could then be further evaluated by direct identification and direct susceptibility testing. Evaluation of this modification has been reported by Jenkins et. al. (3) Heinze et. al. (2), and Thrupp (8). If the Autobac determination is negative after 3 h, a 5- or 6-h reading would still be required to detect some of the slower-growing organisms. Regardless of the procedure, it would be important to inoculate conventional media in special situations when patients are on antibiotic therapy or when low colony counts may be significant.

With these considerations, the Autobac procedure can be effectively used as a method for processing urine specimens in clinical laboratories.

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