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Rapid Semiautomated Screening and Processing of Urine Specimens

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A rapid urine culture procedure was evaluated in which positive urines were detected by using light-scatter photometry (Autobac). Specimens were analyzed at 3, 5, and 6 h. Specimens detected as positive at 3 h were then further evaluated by a direct 3-h susceptibility procedure (Autobac) and by a 4-h identification procedure (Micro-ID). Of 949 specimens, 175 had $>10^5$ colony-forming units per ml by colony count. Of these latter specimens, 75.4% had been detected by 3 h, and 95.4% were detected by 6 h. Of specimens positive by Autobac at 3 h, 96% (95.7%) had $>10^5$ colony-forming units per ml. If pure by Gram stain, those positive specimens were inoculated to direct susceptibility and identification systems. When direct Autobac susceptibilities were compared with the standard Autobac method done from the plate the following day, discrepancy rates were 1.3% very major, 2.1% major, and 7.4% total. The direct identifications were 94% (94.2%) correct when using the Micro-ID manual and a collection of octal patterns unique to this system, in which urine/broth culture inoculum was employed instead of the usual organism colony suspension. Those urine specimens negative after screening at 3 h were evaluated at 5 and 6 h, and an additional 126 specimens were detected as positive. These were then processed by routine plate inoculation, due to the limitations of the work day. By 6 h, 95.4% of specimens with $>10^5$ colony-forming units per ml were detected. The 4.6% false-negative results consisted of patients on antibiotics, or slowly growing bacteria suspected of being distal urethral contaminants. Thus, 83.5% of the urine cultures received by 9:00 a.m. (10.6% 3-h positives and 72.9% negative at 6 h) could be evaluated and reported within one 8-h work day.

Several studies have been reported recently describing screening methods for bacteriuria. Most of these screening methods generate preliminary reports which establish the existence of infection without providing organism identification or antibiotic susceptibility. Thrupp et al. (9) proposed a urine culture technique which included screening for bacteriuria with light-scatter nephelometry, rapid direct gram-negative bacilli identifications using Pathotec strips (General Diagnostics, Division of Warner-Lambert Co., Morris Plains, N.J.), and direct antimicrobial susceptibility testing using light-scatter photometry (Autobac-Pfizer Diagnostics, New York, N.Y.). We have attempted to evaluate and modify that system to provide a practical clinical microbiology laboratory method.

MATERIALS AND METHODS

Specimens. The Clinical Microbiology Laboratory at the University of Utah Medical Center supplied 949 urine specimens, of which 549 were a collection of routine urine culture specimens obtained over an 8-week period. The remaining 400 specimens were collected from a group of catheterized patients. Specimens from the latter group were collected after insertion of an indwelling Foley catheter and then daily as part of an ongoing catheter study. These catheter specimens were rejected from the screening study once they contained a colony count of greater than 10^5 colony-forming units (CFU) per ml, to avoid positive specimen repetition. All specimens were refrigerated at 4°C after initial processing by the Clinical Microbiology Laboratory, and were saved for screening the following morning. Specimens older than 24 h were rejected. A repeat plate quantitation was carried out in conjunction with the urine screening procedure.

Instrumentation. The instrument used to measure light scatter was the Autobac, a system comprised of a photometer module and an incubator/shaker designed to rotate 30 cuvettes at a specific speed (220 rpm) and temperature (37°C). Light-scatter photometry measured turbidity by converting the light scatter by the organisms in suspension into a measurable voltage reading. An increase in turbidity correlated with an increase in light scatter, resulting in a decrease in the voltage reading.

Quantitative urine culture. All urines were streaked with a 0.001-ml calibrated loop on to sheep blood agar and MacConkey agar plates, and a colony count was read at 18 to 24 h (2). These agar plates

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were inoculated within 1 h of the time that the Autobac cuvette was inoculated with the same specimen.

Screening procedure. For the purposes of this study, 10^5 CFU/ml was considered a significant colony count as shown as Kass (5). The screening of urines by Autobac was accomplished by inoculating a broth chamber with urine and observing for a change in voltage at timed intervals. As described by Thrupp et al. (9), a positive urine is detected when the photometer voltage drops more than 0.2 V.

The Autobac cuvette was filled with 18 ml of low-thymidine Eugonic broth, resulting in each chamber receiving approximately 1.4 ml of broth. Each urine specimen was pipetted into two cuvette chambers in amounts of 0.1 and 0.2 ml. The cuvettes were placed in the 37°C incubator/shaker and rotated at 220 rpm. After incubating initially for 15 min to equilibrate the temperature, and to provide a more homogeneous mixture to dissolve crystalline material, each cuvette was then read on a photometer in the "calibrate mode" for a base-line reading. Additional readings were done at 3, 5, and 6 h. The urines found to be positive after 3 h of incubation were assessed for purity by a Gram stain and subcultured immediately to a rapid direct identification and a rapid antibiotic susceptibility system. Those urines not positive at 3 h were reanalyzed at 5 and 6 h by the photometer.

Gram stain. Gram stain reagents were prepared in accordance with Hucker's modification (6). All urines positive by Autobac screen at 3 h were Gram stained for a check of purity. Predominantly pure stains were defined as those in which an oil immersion field full of the predominant organism contained less than 10 organisms of a different morphology.

Direct antimicrobial susceptibilities. Direct antimicrobial susceptibility tests were performed on all cultures positive after 3 h of incubation and for which the Gram stain was pure or predominantly pure. A few drops of the turbid screening broth were removed by sterile pipette to inoculate a buffered-saline tube, which was standardized on the photometer to the proper range of turbidity. The susceptibility procedure was performed according to the manufacturer's instructions, using low-thymidine Eugonic broth (7, 8). A sheep blood agar plate was inoculated from the cuvette growth to assess specimen purity. A routine Autobac susceptibility test was performed the following day using organisms obtained from the purity check plate or the sheep blood agar quantitation plate of that specimen. The antimicrobial agents used are recorded in Table 1.

Identification procedure. Micro-ID (General Diagnostics) was used for the identification of gram-negative bacilli. Micro-ID is a 15-test biochemical strip made specifically for a 4-h identification of *Enterobacteriaceae* (1). The standard method for Micro-ID identification was modified by using several drops of the turbid screening broth and inoculating a 4-ml tube of saline (0.85%) to reach a McFarland no. 0.5 standard (6). Occasionally, additional time was needed to provide a sufficiently turbid suspension, after photometer analysis gave a positive reading, to gain the required inoculum for the Micro-ID method.

A repeat Micro-ID strip was inoculated the following day with organisms from the purity or quantitation plates by using manufacturer's recommended procedure. Organisms were identified by interpreting the test strip reactions and converting the results to an octal number. The identification manual used was the preliminary edition published in March 1978. The standard identification used for comparison was a set of classical biochemical tests used by the clinical laboratory. Those included triple sugar iron agar, lysine iron agar, motility-indole-ornithine decarboxylase medium, Simmons citrate agar, phenylalanine deaminase-urea broth, and, when necessary, Voges-Proskauer, deoxyribonuclease, acetate, and sugar fermentations, as recently described (1). Organisms were identified according to Edwards and Ewing (4).

TABLE 1. *Antibiotics used in susceptibility testing*

For gram-negative rods	Amt (μg)	For gram-positive cocci	Amt (μg)
Trimethoprim/ sulfamethoxazole	18	Ampicillin	4.5
Nitrofurantoin	15	Ampicillin	0.22
Tobramycin	10	Tetracycline	0.5
Tetracycline	1.2	Methicillin	5
Kanamycin	22	Kanamycin	22
Gentamicin	9	Gentamicin	9
Colistin	13	Erythromycin	2.5
Chloramphenicol	4	Clindamycin	2
Cephalothin	15	Ampicillin	4.5
Carbenicillin	120	Penicillin	0.20
		Cephalothin	15

RESULTS

The difference in the rate of false-positive and false-negative results with urine screen inoculum sizes (0.1 or 0.2 ml) was not statistically significant. Therefore, the figures cited in the text will reflect the data using 0.1-ml inocula for the sake of simplicity, unless otherwise stated. (Tables 2 and 3 bear out this conclusion.)

Of the 949 urines processed, 175 were found to contain greater than 10^5 CFU/ml by calibrated loop technique. At 3 h, the false-negative rate (false-negatives divided by the total positives by the calibrated loop technique) (3) was 24.6% (Table 2). Of those specimens positive by loop, 75.4% were detected by screening at that time. After 6 h, the false-negative rate dropped to 4.6%. Of the eight urines (colony counts greater than 10^5 CFU/ml) not detected by Autobac after 6 h, four of the patients were receiving antibiotics to which the organism was susceptible. If one excluded those four urines because the specimen would have been plated for quantitation due to patient therapy, the false-negative rate would be 2.3%. The remaining four urines included two samples containing *Staphylococcus epidermidis*, one with diphtheroids, and one with yeast.

False-positives are shown in Table 3. At 3 h, the false-positive rate (false-positives divided by total negatives by loop) (3) was 1%. At 6 h, the

TABLE 2. *Inoculum differences in false-negatives by Autobac urine screening compared to the calibrated loop method*

Specimens	Time (h)	0.1-ml cuvette inoculum		0.2-ml cuvette inoculum	
		No.	Rate[a] (%)	No.	Rate (%)
Clinical	3	29	21.0	28	20.3
	6	3	2.2	5	3.6
Catheter	3	14	37.8	14	37.8
	6	5	13.5	5	13.5
Total	3	43	24.6	42	24.0
	6	8	4.6	10	5.7
	6[b]	4	2.3	5	2.9

[a] False-negative rate = False-negatives by screening, divided by the total positives by calibrated loop technique (3).

[b] Excluding patients on antibiotics.

TABLE 3. *Inoculum differences in false-positives by Autobac screening compared to calibrated loop method*

Specimen	Time (h)	0.1-ml cuvette inoculum		0.2-ml cuvette inoculum	
		No.	Rate[a] (%)	No.	Rate (%)
Clinical	3	3	0.7	5	1.2
	6	72	17.5	80	19.5
Catheter	3	3	0.8	6	1.7
	6	24	6.7	22	6.1
Total	3	6	0.7	11	1.4
	6	96	12.4	102	13.2

[a] False-positive rate = False-positives by screening, divided by total negatives by calibrated loop technique.

false-positive rate increased to 12.4%. Of the false-positives detected, 49.0% had colony counts above 10^4 CFU/ml, and 79.2% had colony counts above 10^3 CFU/ml. This quantitation would be apparent in the routine laboratory work-up of urines positive by screening at 6 h, since all of these samples would be streaked on sheep blood agar and MacConkey agar plates for overnight incubation and quantitation. Four false-positive results were obtained due to either cuvette leakage between chambers or spill-over during shaking. Only once did this occur by 3 h of incubation.

Table 4 shows a breakdown as to when different organisms became positive. *Enterobacteriaceae* were more frequently detected at 3 h, whereas yeast and gram-positive cocci were significantly slower.

We evaluated the screening detection of all urines that had any growth on quantitative plate cultures to determine the recovery rate, primarily at 6 h, of those urines with 10^4 organisms per ml. At 6 h, all specimens with greater than 50,000 CFU of *Enterobacteriaceae* per ml were detected from patients not receiving antimicrobial therapy. For the *Enterobacteriaceae*, 77.3% (17/22) of all specimens with 10,000 to 100,000 CFU/ml, and 89.5% of those with 12,000 to 100,000 CFU/ml, were detected. If one also includes potential contaminants and non-*Enterobacteriaceae* isolates, only 58.8% (47/80) of the total of all organisms present at over 10,000 but less than 100,000 CFU/ml were detected. The recovery for specific groups of organisms, present at over 10,000 but less than 100,000 CFU/ml, was as follows: yeast, 41.7% (5/12); *Staphylococcus*, 40% (4/10); streptococci, 66.7% (14/21), with most being enterococcus detected in this range; diphtheroids, 55.6% (5/9); and *Pseudomonas*, 66.7% (2/3). Three patients from whom 10^4 to 10^5 CFU of *Enterobacteriaceae* were isolated per ml were receiving antimicrobial agents and are not in the above statistical tabulations. The system failed to detect each of these three; however, they were identified because of the policy of plating all specimens from patients receiving antimicrobial agents.

Purity Gram stain. Of the 138 urines positive at 3 h and stained for purity, 118 (85.6%) were found to be pure or predominantly pure. Of these, 101 were gram-negative rods, 16 were gram-positive cocci, and 1 was comprised of diphtheroids. The Gram stain culled 86.3% of the mixed cultures.

Antibiotic susceptibilities. Direct antibiotic susceptibility tests were run on 116 urines found positive at 3 h. Of these 116, 95.7% (represents a 1% false-positive, using the definition given above) had colony counts in excess of 10^5 CFU/ml, and all had colony counts in excess of 10^3 CFU/ml. The following day, 126 susceptibility tests were repeated for comparison. The 10 additional organisms were morphological variants, with different antibiotic susceptibility patterns, found on quantitation plates in excess of

TABLE 4. *Timing of the detection of urines having greater than 10^5 CFU/ml*

Organism	% Positive after incubation periods of:	
	3 h	6 h
Enterobacteriaceae	89.6	97.9
Pseudomonas sp.	70.0	90.0
Gram-positive cocci	60.7	89.3
Diphtheroids	33.3	83.3
Yeast	0	87.5
Totals	75.4	95.4

10^5 CFU/ml. The discrepancy rates for gram-negative bacilli were 1.5% very major, 2.3% major, and 4.4% minor. For purposes of definition, we have assumed that the repeat susceptibility test is the standard for comparison, since it was performed on a known pure culture. A very major discrepancy is a change, on repeat susceptibility testing, of a result from susceptible to resistant, and a major discrepancy is a change from resistant to susceptible. A minor discrepancy is any change to or from an intermediate susceptiblity value. The reader should bear in mind that the repeat test in this instance was the "standard," assumed more likely to be "correct." Gram-positive cocci gave 0% very major, 1.6% major, and 2.6% minor discrepancy rates. Three antibiotics, chloramphenicol, cephalothin, and nitrofurantoin, accounted for 62.9% of the major and very major discrepancies for gram-negative bacilli. Gentamicin, cephalothin, and erythromycin accounted for 100% of the major and very major discrepancies for gram-positive cocci.

Of the 116 urines eligible, after Gram-stain analysis for direct susceptibility testing, 9 were subsequently found to contain at least two organisms with colony counts greater than 10^5 CFU/ml. The susceptibilities of the more rapidly growing organisms were expressed. The slower-growing organisms included: enterococci (6), *Pseudomonas* (1), staphylococci with a *Proteus* (1), and *Proteus mirabilis* (1). Since the susceptibility patterns of gram-positive cocci cannot be correlated with those of gram-negative bacilli, the susceptibilities of the slower-growing organisms were not incorporated into the percentage correlation figures. Of the nine mixed cultures, five were recognized in the purity Gram stain but were set up because of a single organism predominance. If one were to exclude these, 3.4% of the susceptibilities reported would have been from specimens on which we did not recognize a mixture of two or more significant organisms.

Identification. A Micro-ID identification was performed on 101 urines found positive with gram-negative rods at 3 h. The following day, 109 identifications were performed, which included the morphological variants found in excess of 10^5 CFU/ml on the sheep blood agar or MacConkey agar plates from calibrated loop streaking, and also included one culture which contained two different gram-negative rods. Of the direct identifications, 95.0% were done on urines with colony counts exceeding 10^5 CFU/ml.

A problem was encountered when using the Micro-ID kits, in that the buffering action of the Eugonic broth with which the Micro-ID saline inoculum was made caused interference in several biochemical reactions. These included the sugar fermentations, *o*-nitrophenyl-β-D-galactopyranoside, and indole production. Though characteristic reactions resulted from this interference, the octal patterns keyed out incorrectly in the identification manual. A correct key-out was obtained only 58.4% of the time, using the identification manual. However, using additional octal patterns that were unique to this system and which were recognized as being characteristic of certain organisms, 94.1% of the isolates were identified correctly. (These octal patterns are: for *Escherichia coli*, 20037, 20417, 20437, 20137, 21417, 21436, 21451, 22037, 23437, 62431, and 63471; for *Klebsiella pneumoniae*, 60600 and 60660; for *Proteus rettgeri*, 32100; and for *Pseudomonas aeruginosa*, 01700, 21700, and 21100.) They include characteristic patterns for *P. aeruginosa*, an organism not included in the identification manual. We have subsequently eliminated this problem by using centrifuged portions of the urine sample to obtain the Micro-ID inoculum.

Of the 101 urines that received direct identification, 8 contained at least two organisms, each of which had colony counts exceeding 10^5 CFU/ml. Four of these were detected by the purity Gram stain. The gram-negative bacillus was identified correctly in six of the eight when using the identification manual, and in eight of the eight when using the new octal patterns.

The Micro-ID, when done on "day 2" from the quantitation plates, gave the correct identification 95.4% of the time as compared to the conventional identification method.

DISCUSSION

Based on the screening results, a 3- and 6-h reading on the Autobac can be used as the screening portion of a laboratory urine processing method, provided that the urine specimens arrive in the laboratory in a timely fashion. The 3-h reading provides a low false-positive rate, and those 3-h positive urines have a high probability of containing a single organism in significant numbers. By including a 6-h reading, there is assurance that 95% of the positive urines are detected. Those not detected are more likely to contain organisms frequently viewed as questionable pathogens, or to come from patients receiving antibiotics, who would, under our proposed protocol, have urines streaked to primary isolation (sheep blood or MacConkey agar) plates. Urines negative at 6 h can be reported as negative with a high degree of reliability. A recommended procedure would be to perform rapid direct identifications and antibiotic susceptibility tests on 3-h positives. Urines positive at

6 h would be plated out routinely from the original refrigerated urine and worked up with rapid identifications and susceptibilities, if warranted, the following day. In situations where there is a low incidence of positives, such as in screening large asymptomatic populations, it might be practical to use only a 6-h Autobac reading.

This screening method is designed to detect urines containing high colony counts. In that light, urines from patients in which lower colony counts may be significant should be processed routinely, in addition to this rapid method. These patients include those on antibiotics, those with urinary catheters, and those from whom suprapubic aspirates were used for specimen collection. The primary reason to process routinely urines from patients on antibiotics is that the antibiotics may prevent growth in the screening broth, yet allow growth when dispersed on the surface of solid media. A total of 50.0% of the false-negatives at 6 h in screening by Autobac occurred in patients receiving antibiotics, including both of the false-negative *Pseudomonas* isolate specimens.

Many physicians and microbiologists might have concern for a system in which specimens with numbers approaching 10^5 might be missed. The screening procedure picked up at 6 h all *Enterobacteriaceae* over 50,000 CFU/ml and 89.5% of the *Enterobacteriaceae* over 12,000 CFU/ml, with a similar recovery for *enterococcus*. Two of three *Pseudomonas* isolates in the 10^4 to 10^5 range were detected. Recovery figures were not as high for non-enterococcal streptococci, staphylococci, and yeasts.

The false-negative rate will drop to less than 3% if all urine specimens from patients receiving antimicrobial agents and which are negative by urine screen are plated out after the 6-h reading. The organisms that then remain in the false-negative category are almost all perineal contaminants. Significant pathogens missed by this method were less than 1%.

To minimize the problem of false-negatives due to the presence of antimicrobial agents, the University of Utah Clinical Microbiology Laboratory sends out reminders to physicians who provide inadequately filled out forms. This has improved the amount and quality of information on the slips, so that 85% of slips received are now properly completed.

Of the positive urines evaluated for purity by Gram stain, 90 contained a single organism. The majority of mixed cultures, in which both organisms were observed in significant quantities, contained gram-negative bacilli with enterococci. It was evident that enterococci in significant numbers may be outgrown in the screening procedure prior to the purity Gram stain. In that light, it is recommended that all urines found mixed by Gram stain be processed routinely on quantitation plates to detect the presence of additional organisms with significant colony counts.

The high correlation of direct antibiotic susceptibilities with those suceptibilities done routinely gives confidence that this method can be used in a routine laboratory setting.

The accuracy of the Micro-ID in direct identification of gram-negative bacilli was hampered by the buffering action of the Eugonic broth. Interpreting Micro-ID reactions was difficult at best because the degree of variance from octal patterns listed for appropriate organism identification was dependent on the amount of broth inoculum needed to bring the saline to the McFarland no. 0.5 turbidity. With a turbid screening chamber, only a few drops of broth were necessary for inoculation, and the reactions had no interference. With several urines, when the screening chamber was minimally turbid, 1 ml or more was needed to bring the saline to the McFarland no. 0.5 standard turbidity. It was determined that 0.2 ml of Eugonic broth used in the saline inoculum allowed normal reactions, whereas 0.5 ml of broth used in the saline inoculum was enough to cause abnormal reactions by 4 h.

To correct this problem, we have subsequently explored several alternatives. (i) The organisms can be spun down in the screening broth, and the supernatant broth is then aspirated and replaced with saline. (ii) The laboratorian can delay setting up the Micro-ID until the broth reaches a turbidity wherein a smaller inoculum can be used. (iii) Any specimen with an octal number not keyed to an anticipated urinary pathogen can be allowed to incubate longer. It was found that by 16 h, all of these non-organism-related patterns had reverted to normal, except those in which at least 2 ml of Eugonic broth was used in the saline inoculum. (iv) The last alternative would be to use an identification method in which the buffering activity of the broth was considered in creating the octal code listing, and, therefore, would not affect the identification. Any one of those alternatives could offer high-accuracy identifications.

With the urine processing system, 10.6% of the urines (57.7% of all significant positives) received a same-day identification and antimicrobial susceptibility test. An additional 72.9% of the urines were negative by screen after 6 h. Thus, 85.5% of the workload was completed within approximately 8 h. This figure dropped to 82.3% when we used the recommended method of working up only urines with pure Gram stains. In that

context, the urine processing method offers a considerable time and effort savings for the clinical microbiology laboratory and a marked decrease in the amount of time between specimen collection and final laboratory result reporting.

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